VALIDATION AND APPLICATION OF INFRARED THERMOGRAPHY FOR THE ASSESSMENT OF BODY CONDITION IN PINNIPEDS

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ABSTRACT

Infrared thermography (IRT) was used to collect baseline information on skin surface temperatures of two species of pinnipeds, the harbor seal (*Phoca vitulina*; n = 6) and the Steller sea lion (*Eumetopias jubatus*; n = 2). The IRT technique was validated against objects of known temperature and through post-collection software manipulation of environmental parameters that influence IRT output (emissivity, distance, relative humidity, ambient temperature and reflected temperature). From February 2007 to February 2008, biweekly measurements were taken of skin surface temperature (FLIR P25 infrared camera) with subsequent measurements of blubber depth (SonoSite Vet180 portable imaging ultrasound system) on captive individuals at the Alaska SeaLife Center, Seward, Alaska. Once validated, skin surface temperatures in 10 defined regions (whole body, torso, head, eye, muzzle, shoulder, axillae, hip, fore and hind flipper) were used to determine seasonal variability as well as consistent hot or cold spots, and of those spots, which may act as thermal windows (defined areas of active heat loss and/or retention). Concurrent measurements of blubber depth were compared to skin surface temperatures at eight body sites to assess: a) the impact of insulation level on skin surface temperature on a site-specific scale, and b) the potential use of IRT as an alternative method for the non-invasive measurement of body condition. Both species varied seasonally in skin surface temperature from winter to reproductive and molt to winter, however, harbor seals had greater regional variation. Similar hot and cold spots were consistently recognized in both species with shoulder, axillae, fore and hind flipper identified as likely thermal windows. While some site-specific significant relationships were found between skin surface temperature and blubber thickness, insulation level alone explained a very small portion of the variance. Future studies to determine the factors influencing the variance on skin surface temperature (i.e., blood flow to the skin) warrant further exploration.

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GENERAL INTRODUCTION

History of thermography

Infrared thermography (IRT) is a technique used to determine surface temperature by measuring the amount of radiation emitted from an object. The first thermograph was produced inadvertently in 1840 by astronomer Sir John Herschel while in search for a new optical filter material used in telescopes to help reduce the brightness of the sun as well the amount of heat produced by the scope (FLIR Systems, 2004). Eventually, the military adapted the technology for various detection devices in vessel and aircraft operations in the early 1900's. Not until the mid-1950s did IRT become readily available for scientific and industrial applications. Prior to this time, systems were bulky, expensive, required liquid nitrogen for cooling, and functioned only in a horizontal orientation making IRT equipment inconvenient (Jones, 1998). Recent advances offer more affordable, portable, and sophisticated equipment, which has significantly increased the use of IRT for industrial, medical, and veterinary use. In turn, this has provided the opportunity to further explore and understand thermal physiology.

Basic principles

Compared to visible light energy (400 – 700 nm), infrared energy occurs at longer wavelengths (750 nm – 1 μm), and its measurement therefore requires a device that is sensitive to this spectral band. The IRT camera allows us to see infrared energy within the field of view which is then converted into a temperature unit (°C) by the camera's software. Emissivity, reflected temperature, ambient air temperature, distance, and relative humidity are the five parameters required for the software to generate an object's surface temperature reading. The greater the accuracy of the parameter estimates, the closer the calculated temperature of the object is to the actual surface temperature (Orlove, 1982; Hamrelius, 1991).

All objects radiate or emit infrared energy proportional to their temperature, with the degree of infrared emission being proportional to the rate of absorption at the same

wavelength. Although theoretical, a blackbody is an object that is a perfect emitter of infrared energy (i.e., emissivity = 1.0) and is constant at all wavelengths (Orlove, 1982; Speakman and Ward, 1998). An emissivity of 0.95 – 0.98 best describes live animals including humans (Best and Fowler, 1981; Cuyler et al., 1992; Speakman and Ward, 1998; Dunbar and MacCarthy, 2006). Metals, on the other hand, have a much lower emissivity (aluminum ~0.20, lead ~0.28, polished iron ~0.21, stainless steel ~0.35, tin ~0.06), therefore serve as better reflectors than radiators of infrared energy, much like a mirror reflecting visible light (FLIR Systems, 2004). Reflected temperature is an image taken by the IRT camera of the background opposite the object of interest representative of the heat being reflected upon the object of interest. Relative humidity and ambient air temperature are collected at the location of the object. Finally, distance was measured from the object of interest to the lens of the camera.

Of interest to medical, veterinary, and biology fields is the potential use of infrared radiation as a proxy for heat flux in animals. Heat flux is defined as the flow of energy at a given rate of time. Warm areas or those of increased blood flow commonly occur with inflammation, injury, and abscesses. Conversely, cool areas or those of reduced blood flow can be caused by nerve damage or dead tissue (Kastberger and Stachl, 2003). Varying degrees of insulation depth and vascularization of the skin surface will also manifest as warm or cool areas throughout the body, which can remain constant through time or fluctuate due to biological or physiological pressures inflicted upon the animal (Tarasoff and Fisher, 1970; Cena and Clark, 1973; Clark et al., 1977; Bryden and Molyneux, 1978). Most recently, IRT has been utilized as a prognostic tool for the detection of breast cancer, rabies infection, pregnancy, and equine lameness (Eddy et al., 2001; Xie et al., 2004; Dunbar and MacCarthy, 2006; Durrant et al., 2006).

Application to wildlife biology

When collecting physiological data from wildlife, sedation or restraint is often needed. Depending on the type and location of collection method used and animal of interest, this

can become logistically time consuming and expensive. Furthermore, some populations, especially where endangered species are concerned, have such tight permitting restrictions that restraint is not feasible and data collection is limited. To date, most thermoregulatory studies have involved thermocouples, mercury thermometers, thermistors, or temperature-sensitive heat sensors all of which are data collection methods that require physical contact (Irving, 1955; Blix et al., 1979; Boily and Lavigne, 1996). The stress of restraint (and/or sedation method used) directly influences core temperature which will interrupt normal physiologic parameters and therefore thermal data (Bartholomew and Wilke, 1956; Okada et al., 2007). Not only is IRT noninvasive, but it can be used at either close or distant proximities (<1 to >1000 m), thereby reducing and/or eliminating disturbance altogether (McCafferty, 2007).

In zoo and field settings, IRT has become a very popular technique. Animals can be scanned for abnormalities or injuries, allowing for spot assessment prior to handling. IRT is not only advantageous as a diagnostic tool, but is being increasingly used in the research arena to examine physiological responses, such as regional facial temperature changes due to fear (Nakayama et al., 2005), thoracic and pelvic limb temperature changes in response to exercise-generated heat (Simon et al., 2006), and eye temperature changes due to stimulation-induced stress (Stewart et al., 2007; Stewart et al., 2008).

Why marine mammals?

Marine mammals are interesting focal species for the study of thermal physiology because of their amphibious lifestyle. The marine environment offers relatively stable temperatures and relief from convection due to air, evaporation, and the sun's radiation when at depth. However, these animals must deal with water being 25 times higher in thermal conductivity than air (Bartholomew and Wilke, 1956; Noren et al., 2008). Even in air, as animals are subject to variable environmental conditions (i.e., wider temperature range, wind, direct sunlight), this can demand elevated energy expenditure to maintain homeothermy. An example of a way to reduce these energetic costs includes decreased

ambient air temperatures when seals will decrease their skin temperatures to minimize the temperature gradient between the air and skin (Hart and Irving, 1959; Hansen et al., 1995). Marine mammals have adapted to this lifestyle primarily through the use of an insulative blubber layer, in addition to having a thick pelage, counter current heat exchange system and a low surface area to mass ratio.

The harbor seal (*Phoca vitulina)* is a widespread species covering both Atlantic and Pacific oceans, and along the coasts of British Columbia as far as northwest Alaska down to the Baja California (Rice, 1977). An average adult Pacific harbor seal has a body mass of about 80 kg, with males being slightly larger than females (Riedman, 1990). Females from the Pacific population pup on ice or land between mid – May through mid – July (King, 1983). For unknown reasons the populations of Pacific harbor seals in Alaska have been declining since the mid-1970s, especially in the areas of Prince William Sound and the Gulf of Alaska (Riedman, 1990).

The Steller sea lion (*Eumetopias jubatus*) is distributed from the northern California coast through the Bering Sea into Russia. Two distinct populations, the eastern and western stocks are divided geographically at 144 ° W longitude, approximately through the center of the Gulf of Alaska. Steller sea lions give birth to a single pup that is weaned between 1 – 2 years of age and breed on rookeries from mid – May through mid – July (Pitcher and Calkins, 1981; Riedman, 1990). While the eastern stock appears to have remained stable, the western stock has declined by more than 80 % in some areas of Alaska since the early 1970s (Loughlin et al., 1992), and was listed as an endangered species in 1997 (U.S. Federal Register 62:24345 – 24355).

Homeostasis in seals and sea lions is maintained through continual behavioral and physiological adjustments sometimes at an energetic cost, which may decrease body condition especially in periods of molt or breeding. Both harbor seals and Steller sea lions have adaptations for temperature regulation, including a low surface area to mass

ratio, blubber and fur, high rate of metabolism or internal heat production relative to similar sized terrestrial animals, and a countercurrent heat exchange system (Riedman, 1990). While these adaptations are beneficial in the marine environment, they can be disadvantageous while on land (i.e. overheating due to high insulation or hypothermia due to wet pelage (Irving et al., 1962; Blix et al., 1979).

Blubber in pinnipeds is not only used as an insulation mechanism to reduce heat loss, but is a living organ used for buoyancy and energy reserves that fluctuates seasonally (Ryg et al., 1990; Rosen and Renouf, 1997; Mellish et al., 2007) with blood vessels within that prevent the skin surface from freezing. Harbor seal blubber is typically uniform in composition and location, whereas blubber in Steller sea lions is interrupted with layers of connective tissue with some deposition in the muscle fascia (Mellish et al., 2004). A recent ultrasound study by Mellish et al. (2007) showed that harbor seals in general had thicker and more consistent insulation, while both species had maximum blubber depth at the lateral axillae. Furthermore, variations in blubber depth and consistency tracked seasonal and mass related changes in harbor seals, whereas only mass at 3 specific site location changes and no seasonal changes were noted for Steller sea lions. Such differences could result in profound species-differences in heat loss and therefore thermoregulatory tactics. Harbor seals and Steller sea lions exhibit different modes of swimming and therefore use different muscles. This may be related to the variation seen in blubber thickness and possibly heat produced at different sites. Several heat flux studies using IRT in pinnipeds have looked at how and where heat dissipation occurs in these animals (Mauck et al., 2003; Willis et al., 2005). Variable regions of blubber insulation and pelage coverage suggest that there may be areas along the body that preferentially allow for heat dissipation (i.e., thermal windows; Bartholomew and Wilke, 1956). On the other hand, vascularization between species may differ, with the end result being a consistent pattern of heat loss or retention throughout the body versus specific regions of heat flux (Bryden and Molyneux, 1978; Molyneux and Bryden, 1978).

Project description and study animals

The purpose of this project was to assess the application of thermal imaging to wildlife research. More specifically, the study was designed to test the ability of IRT to non-invasively assess health and condition in seals and sea lions (Chapter 3, The thick and thin of body condition: Does blubber depth influence skin surface temperature in pinnipeds?), which first required a description of normal baseline thermal patterns (Chapter 2, Thermal windows in seals and sea lions: what's hot and what's not?). Six juvenile Pacific harbor seals (6F) and two adult female Steller sea lions at the Alaska SeaLife Center (Seward, AK) were the subjects of the validation study of infrared thermography. Data were collected for each individual up to two times per month with a FLIR P25 infrared camera using FLIR ThermaCam Researcher Pro version 2.8 SR-1 for image analysis (FLIR Systems, Danderyd, Sweden), coupled with a non-invasive assessment of blubber depth via a SonoSite 180Vet portable imaging ultrasound (SonoSite, Inc., Bothell, Washington, USA) from eight body sites. Data collection occurred during one annual cycle to evaluate baseline regional thermoregulatory responses related to seasonal changes and to assess the feasibility of using infrared thermography data as a proxy for body condition in pinnipeds.

Thermoregulatory fluctuations due to various factors include the body's response to help maintain homeostasis. Through the use of IRT, we hope to identify areas of active heat loss and/or retention, otherwise known as a thermal window, and then further determine whether site-specific areas of blubber depth correlate to skin surface temperature. A combination of these two imaging tools may provide the opportunity to non-invasively assess the body condition of individuals in two Alaskan pinniped species of concern.

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CHAPTER 2[1]**:** THERMAL WINDOWS IN SEALS AND SEA LIONS: WHAT'S HOT AND WHAT'S NOT?

ABSTRACT

1. Infrared thermography (IRT) was validated to determine the accuracy of surface temperature measured via IRT against objects of known temperature. Manual manipulation of object parameters required for the software for accurate output was also performed.

2. Baseline seasonal variability of regional skin surface temperatures, consistent locations of hot and cold spots, and potential thermal windows were identified. Images were collected in six juvenile female harbor seals (*Phoca vitulina*) and two adult female Steller sea lions (*Eumetopias jubatus*) at the Alaska SeaLife Center, Seward, AK.

3. Ten regions of interest were identified (whole body, torso, head, eyes, muzzle, shoulder, axillae, hip, fore and hind flipper). Seasonal variation was observed from winter (Oct – Apr) to reproductive (May – Jul) and molt (Oct – Apr) to winter ($p < 0.05$). These patterns were more apparent in the harbor seals than in the Steller sea lions. By contrast, spatially and temporally consistent hot and cold spots suggested that shoulder, axillae, fore and hind flipper may act as thermal windows in both species.

Keywords: Thermoregulation, infrared thermography (IRT), Steller sea lions, harbor seals, *Eumetopias jubatus, Phoca vitulina,* thermal windows

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INTRODUCTION

Infrared thermography (IRT) is a non-invasive method increasingly used in veterinary and wildlife research applications as a diagnostic health tool (i.e., McCafferty et al., 1998; Spire et al., 1999; Eddy, 2001; Phillips and Heath, 2001; Dunbar and MacCarthy, 2006; Simon et al., 2006). In marine mammals, IRT has been applied to studies of heat flux (dolphins - Williams et al., 1999; Steller sea lions - Willis and Horning, 2005), characterization of thermal windows (whales – Cuyler et al., 1992; seals - Mauck et al., 2003), and in aerial surveys (walruses - Burn et al., 2006). Remarkably lacking in these studies are the essential technical and species-specific validation of the method, including documentation of the capabilities of the equipment used, influence of parameters (i.e., emissivity, ambient air temperature, distance, relative humidity, reflected temperature) on temperature estimates, and species-specific baseline surface heat patterns.

Thermoregulation in pinnipeds has been studied to understand how homeotherms can function in both an aquatic and terrestrial environment (Bartholomew and Wilke, 1956; Irving et al., 1962). The physical processes of heat conduction, convection, radiation and evaporation influence an animal's ability to maintain thermal homeostasis (Folkow and Mercer, 1986). Blubber is an important source of insulation for pinnipeds, as water has 25 times higher specific heat capacity than air (Bonner, 1984; Ryg et al., 1990; Rosen et al., 2007) yielding a higher rate of heat loss when in water of the same temperature. These animals need to stay warm while in their aquatic environment, yet be flexible in

their ability to maintain or release heat quickly when moving into air, which can be at a higher or lower temperature than water.

Passive sources of heat loss are in areas of the body that have minimal insulation, such as the extremities or furless regions (Irving et al., 1955; Kvadsheim and Folkow, 1997) while active sources of heat loss are due to vascularization, as in the flippers of seals (Bryden, 1978; Bryden and Molyneux, 1978; Molyneux and Bryden, 1978). However, even un-insulated extremities can conserve heat via counter-current heat exchangers (Scholander and Schevill, 1955) by cooling arterial blood before it reaches the extremities keeping the appendage cold to preserve body heat. Fur and hair can also absorb, or reflect solar radiation away from the skin surface (Cena and Monteith, 1976). In marine mammals, thermal windows, defined as sites of active heat dissipation and/or retention, have been identified for some species, including peripheral sites (i.e., dolphin fluke) that are consistent in location through time (Noren et al., 1999; Meagher et al., 2002) and transient location areas along the trunk of some seals (Mauck et al., 2003). However, thermal windows have not yet been defined in the harbor seal or Steller sea lion. Once baseline hot and cold patterns are identified under controlled physiological and environmental conditions, IRT may be used as a diagnostic tool to identify irregular heat loss, possibly due to poor body condition, as well as more acute issues such as potential sites of infection, parasitism or inflammation.

With the decline of Alaskan harbor seals (mid-1970s) and endangered status of the Western stock Steller sea lions (1990), non-invasive methods of health assessment are valuable tools in limiting future disturbance for monitoring purposes in these populations. Therefore, this study addressed four primary questions:

1. How does surface temperature of an object measured via infrared thermography compare to actual temperature of the object in a water bath measured by a standard mercury thermometer?
2. What are the sensitivities of IRT-based surface temperature estimates to errors in parameters that are known to affect IRT (i.e., emissivity, relative humidity, distance, ambient air temperature and reflected temperature)?
3. How are baseline annual surface temperature variations characterized in harbor seal and Steller sea lion body regions, as measured by infrared thermography?
4. Can consistent hot and cold spots be identified in harbor seals and Steller sea lions by infrared thermography within individuals and over time and if so, of these, which can be considered thermal windows?

MATERIAL AND METHODS

Animals and equipment

Six female juvenile Pacific harbor seals *(Phoca vitulina)* and two adult female Steller sea lions *(Eumetopias jubatus)* housed at the Alaska SeaLife Center (ASLC) in Seward, AK were the subjects for the year-long study (Table 2.1). Three juvenile harbor seals, captured from central Prince William Sound and eastern Kenai Peninsula, AK, (PVAT,

PVQI and PVSU) were brought to the ASLC as newly weaned pups in 2004. The other three juvenile harbor seals (PVSH, PVSI and PVTI) were brought to the ASLC (from the same area as the 2004 cohort) as newly weaned pups in 2005. The two adult female Steller sea lions, EJKI and EJSU, were both captured as pups at Maggott Island, Canada in 1993.

Due to a simultaneous long-term nutritional study, the harbor seals were on a lipid-regulated diet of either high fat Pacific herring (*Clupea pallasii*; PVAT, PVQI, PVSI) or low fat Atlantic herring (*Clupea harengus*; PVSU, PVSH, PVTI) mixed with pollock (*Theragra chalcogramma*), capelin (*Mallotus villosus*), and squid (*Loligo opalescens*) (L. Polasek, unpublished data). The two Steller sea lions were fed a daily diet of pollock and Pacific herring supplemented with pink salmon three days a week. All diets included a daily multivitamin dosage based on individual mass (Mazuri 5# Marine Mammal Tablet).

In order to minimize error in data collection, only two investigators (JN, JT) collected thermal images during the year-long study. All thermograms were taken with a FLIR P25 infrared camera (FLIR Systems, Danderyd, Sweden) using an uncooled focal plane array microbolometer sensor. The camera was factory calibrated to an absolute accuracy of ± 2°C, with thermal sensitivity of <0.10 at 30 °C. Infrared images had a resolution of 320 x 240 pixels. Relative humidity (%) and atmospheric temperature (°C) were taken at each event with a Sper Scientific Ltd #850070 Mini Environmental Quality Meter (Sper Scientific Ltd, Scottsdale, Arizona, USA), absolute accuracy of the parameters were ±

6% and ± 1.2 °C, respectively, while resolution was to the nearest 1/10 of a percentage point or degree. A standard mercury thermometer with an absolute accuracy of ± 2 °C and resolution to the nearest 1/10 of a degree, which was placed in a water bath, was used to collect the temperature of the control source (Comark USA, Beaverton, OR, USA). Sea water temperature data were taken from daily aquarium husbandry staff records (Hach Model HQ30d, Hach Co., Loveland, CO, USA). Body mass was collected on the day of the session by mammal husbandry staff (Transcell Model TI-500-SL, Accurate Scales, Terre Haute, IN, USA)

Thermal images were collected two times a month from February 2007 to February 2008. The number of samples varied per month and was dependent upon animal cooperation and concurrent research needs (Table 2.1). Depending on the husbandry and research requirements of the captive animals, images were either taken indoors under artificial fluorescent lighting or in an outdoor exhibit under natural lighting conditions. Indoor images were taken on either a concrete flooring covered with an epoxy coated paint or an aluminum surfacing. A typical session involved moving the animal from a holding area to the room where images took place (approximately 15 meters). For standardization purposes, all thermograms were taken of wet animals, within 5 – 10 minutes of being trained to haul out. Animals were behaviorally controlled to station and moved between images while the thermographer stood in a fixed position. Eight images were taken per animal per session, including right lateral, left lateral, high anterior, low anterior, high posterior, low posterior, ventral, and a reference temperature image. Right lateral, left

lateral, low anterior, low posterior and ventral images were taken at an approximate 90° angle between camera lens and animal. High anterior and high posterior images were taken at an approximate 45° angle between camera lens and animal. The reflected temperature image was taken at a 180° turn away from the animal. Distance between animal and camera was set such that the animal would fill the frame of the picture from tip of tail to tip of nose. Images were taken in gray color palette for ease of focus and increased contrast. The total session time was approximately 10 minutes per animal. No anesthesia or sedation was required.

Validation of IRT

In order to validate IRT to measure skin surface temperature and to determine how parameters influenced temperature estimates, two comparisons were completed. In the first study, two objects of known emissivities (ε) were used, a glass jar filled with water (ε = 0.92) and a stomach temperature radio transmitter pill made of epoxy (ε= 0.84). Both objects were placed in a heated/refrigerated circulator (accuracy of ± 0.5 °C, VWR International, Inc., West Chester, PA, USA) at 5 °C temperature increments (temperature range: 0 – 30 °C). The objects were in the circulator for greater than five hours at each 5 °C increment in order to assure that the entire object was at that temperature. Once at temperature, one IRT image of each object was taken at each 5 °C increment. The second study was completed to assess environmental parameter (emissivity, reflected temperature, ambient air temperature, distance, relative humidity) influence on calculated surface temperature. Each parameter was artificially manipulated within the software on

a single image (with all other parameters constant) for each species (Table 2.2). Emissivity was increased and decreased by 0.02 increments as this is the common range of emissivity values used in living skin tissue IRT study (0.95 – 0.99; Best and Fowler, 1981; Speakman and Ward, 1998). All other parameters were increased and decreased by 10%.

Skin surface patterns

All images analyzed and temperature output was used with the FLIR ThermaCam Researcher Pro 2.8 SR-1 software (FLIR Systems, Danderyd, Sweden). For ease of thermal window visualization, a rainbow color palette was used for analysis. Software corrected parameters included emissivity, ambient air temperature, distance, relative humidity and reflected temperature. One image set was chosen at random (August 22, 2007) for one harbor seal (PVQI) and one sea lion (EJSU) to compare symmetry in surface temperature patterns among the eight image angles. With the exception of biopsy and/or injection sites, thermal patterns were equally visible amongst all positions. Due to the symmetry of thermal patterns, the right lateral image was chosen for analysis and statistical purposes. All other images were used as references only.

This study was not focused on absolute skin temperatures or the size of specific areas of interest, but rather the relative skin surface temperature variation within a region and by season. Therefore, thermal images were subdivided into the following regions for temperature analysis: whole body, torso, head, eye, muzzle, shoulder, axillae, hip, fore

and hind flippers (Figure 2.1). Whole body included the entire animal while torso excluded the head and flipper regions. Regions other than whole body, torso and head, were further thermally identified as hot or cold spots. These regions were chosen based on continued visual appearances in individual thermal images. Hot spots were defined as areas along the body of low insulation and/or high vascularization that could explain regions of high heat dissipation (i.e., areas of high surface temperature). Hypothesized hot spots included the eye, muzzle, shoulder, axillae and hip regions for both harbor seals and Steller sea lions. Cold spots were defined as areas along the body that may be of high insulation and/or low vascularization which could explain low heat dissipation (i.e., areas of low surface temperature). Hypothesized cold spots consisted of both flippers in seals and sea lions. These anatomical and functional definitions for hot and cold spots were considered hypotheses to be tested and later determined if any of these spots were true thermal windows, which are active areas of heat retention and dissipation. Mean temperatures were used for the whole body, torso and head regions as these were regions of the body that included areas differing in insulation and vascularization. For heterogeneous areas containing hypothesized hot spots with minimal or no insulation that require a high level of perfusion for maintenance of normal physiological processes (eye and muzzle), as well as other hypothesized hot spots with little insulation (shoulder, axillae and hip regions), maximum temperatures within defined areas were used for analyses. Minimum temperatures within defined areas were used for hypothesized cold spots (fore and hind flipper) in data analysis.

Data analysis

For analysis of seasonal trends, seasons were defined as winter (Oct – Apr), reproductive (May – Jul) and molt (Aug – Sep), as per Pitcher (1986) and Mellish et al. (2007). Sexual maturity in harbor seals has been shown to be reached between 4 – 9 years (Pitcher and Calkins 1979), therefore our subject individuals were not anticipated to experience a true reproductive season, however, this analysis was provided to maintain consistency with that presented for adult female Steller sea lions. To avoid unequal sample replication, all individuals were represented by using the first data point of each month for annual analyses. To avoid bias due to unequal length of seasons, results were reduced to four consecutive image sessions per animal closest to the center date for each season.

Calculations and statistical analyses were completed using SYSTAT 10 (SYSTAT Inc., Chicago, IL, USA) and plots using SigmaStat 3.5 (Systat Software, Inc., Germany). A simple regression was used to describe the relationship between the known object surface temperature as measured via IRT and as measured via a mercury thermometer. The coefficient of variation (COV) was used to determine variability between body regions. Unlike all other statistical tests used during analyses, COV data were looked at in the winter season only, where six consecutive image sets were used per individual within the same months. Changes in mass and regional skin surface variation across seasons were analyzed by multiple runs of the non-parametric, Mann-Whitney U-test. Water temperature influence on skin surface temperature was analyzed using repeated measures

multivariate analysis of covariance (MANCOVA). Consistent hot and cold regions were identified using a Wilcoxon signed rank test, followed by a manual ranking of means per region to determine the order of hottest to coolest region. Data are presented as mean ± standard deviation (SD) unless otherwise stated. Significance was set at a 95% confidence interval.

RESULTS

Technical parameters

Temperatures estimated via IRT had a strong direct relationship with the temperature measured by the mercury thermometer for the glass jar (IRTglass = 0.962*waterT + 0.641, $r^2 = 0.993$, $p < 0.001$) and the stomach pill (IRTpill = 0.894*waterT + 1.249, $r^2 = 0.999$, $p < 0.001$) (Figure 2.2). This difference was within the absolute accuracy indicated for both measurement techniques (IRT and mercury thermometer), and the absolute estimates are therefore indistinguishable. Since the relationship between IRT and mercury thermometer based temperature estimates are highly linear, IRT estimates were not corrected prior to analysis in this study.

Software parameter manipulation (Table 2.2) changed average surface temperature output of the body image as a whole by a ± 0.1 °C, with some parameters having no change in surface temperature output at the modified increase and/or decrease (harbor seals: ambient air temperature and distance; Steller sea lions: ambient air temperature, distance and reflected temperature).

Changes in body mass

Overall, harbor seal annual mass was 32.3 ± 6.5 kg, minimum mass occurred at molt (29.8 ± 6.1 kg) and maximum mass occurred in winter (33.7 ± 6.5 kg). Harbor seal body mass changed from winter to reproductive ($U = 423.0$, $p = 0.005$), and from winter to molt ($U = 437.5$, $p = 0.002$). There was no significant change from reproductive to molt. In contrast, sea lion body mass did not vary seasonally.

Surface temperature trends

General surface temperatures (whole body, torso and head), maximum surface temperatures (eye, muzzle, shoulder, axillae and hip), and minimum surface temperatures (fore and hind flippers) over the year were averaged by species and are shown in Figure 2.3. Surface temperatures of the whole body and torso in harbor seals were 10.2 ± 1.2 °C and 10.0 ± 1.2 °C, respectively. Steller sea lion surface temperature was 10.5 ± 0.7 °C for whole body and 10.3 ± 0.6 °C for torso. Both regions were highly correlated in each individual over the year (individual harbor seals at or above $r \geq 0.790$; individual Steller sea lions at or above $r \geq 0.996$), therefore only the torso region will be discussed henceforth.

Skin surface temperature variability differed by body region and with species (Table 2.3). In harbor seals, the least variable thermal region throughout the annual cycle was the hind flipper (COV = 5.8) while the most variable region was the shoulder (COV = 12.3). In sea lions, the least variable region throughout the year was also the hind flipper (COV =

2.7), while the head was the most variable (COV = 8.3). Annual fluctuations in seasonal surface temperature by region are shown in Figures 2.4 and 2.5.

Hot and cold regions

Seasonal variation in surface temperature was analyzed by pairwise comparisons between each season using multiple runs of the Mann-Whitney U test (Appendix A). Between winter and reproductive, all regions except for the eye, muzzle and hind flipper demonstrated seasonal surface temperature variation in harbor seals ($U = 84.5 - 191.0$, $p < 0.05$). Between reproductive and molt, the muzzle significantly changed ($U = 175.0$, $p = 0.02$) while all other regions were not significant. Between molt and winter, all surface temperature in regions were significantly different ($U = 65.5 - 122.0$, $p < 0.001$) except for the eye region. Over all seasons, the eye region was the hottest spot (24.2 ± 1.2 °C), while the coolest spot was the hind flipper (6.6 ± 0.7 °C). Seasonal effect in surface temperature was not as apparent in the Steller sea lions as with the harbor seals. Between winter and reproductive, significantly different regions included only the torso, shoulder, hip and fore flipper ($U = 8.5 - 12.0$, $p < 0.03$). No regions differed between reproductive and molt measurements, and only shoulder changed in skin surface temperature between molt and winter ($U = 13.0$, $p < 0.05$). In the sea lions, the overall hottest spot was the eye (25.1 ± 0.3 °C) while the lowest mean surface temperature was the fore flipper region ($7.9 \pm <0.1$ °C).

Seasonal effects in water temperature and hind flipper skin surface temperature of both harbor seals and sea lions are seen in Figure 2.6. Water temperature was determined to not be a driving factor in skin surface temperature ($F_{(1,14)} = 0.003$, $p = 0.956$), however there was a seasonal effect ($F_{(2,14)} = 9.495$, $p = 0.002$) in skin surface temperature.

In the harbor seals, all regions within the winter were significantly different from each other ($p \leq 0.002$) except for shoulder *vs.* axillae and hip and axillae *vs.* hip. In reproductive, all regions were significantly different ($p < 0.05$) except for shoulder *vs.* axillae and axillae *vs.* hip. During molt, all regions varied significantly ($p < 0.05$). In Steller sea lions, all regions varied amongst each other during winter ($p < 0.02$) except for eye *vs.* muzzle. In reproductive, all regions were significantly different from each other ($p < 0.05$) except for whole body *vs.* torso, fore flipper, and hind flipper, head *vs.* hip, shoulder *vs.* axillae and hip, axillae *vs.* hip, and fore flipper *vs.* hind flipper. During molt, all regions were significantly different from each other ($p < 0.05$) except for eye *vs.* muzzle, shoulder *vs.* axillae, and shoulder *vs.* hip.

To determine whether hot and cold spots were consistent across seasons, morphological areas were manually ranked within each season. Areas were ranked from 1 – 10, 1 being the hottest spot and 10 being the coolest spot (Table 2.4). In the harbor seals, the eye region was consistently the hottest spot within each season, while the hind flipper was the coolest spot within each season. In the Steller sea lions, the eye region was the hottest

spot in reproductive and molt, while the muzzle was the hottest spot in winter. The coolest spot was the hind flipper within each season.

DISCUSSION

Technical validation

Five environmental parameters are necessary for accurate IRT based surface temperature estimates: emissivity, ambient air temperature, distance, humidity and reflected temperature. Although the surface of most animals studied to date covers only a small range of emissivity values (0.95 – 0.98, Speakman and Ward, 1998), changes of this entire range result only in minimal changes of surface temperature estimates (refer to Table 2.2).

Most IRT studies involve the use of thermocouples to assess the accuracy of the camera and to accurately predict absolute temperatures (Donohue et al., 2000; Meagher et al., 2002). Because this study was based on relative patterns rather than absolute temperatures, correction factors were not employed, specifically due to the highly linear relationship between water temperature and IRT-based temperatures of the glass jar and stomach temperature radio transmitter pill (Fig. 2.2).

IRT considerations for field settings

While this study focused on validation of IRT in a controlled setting and within a small emissivity range, using this method while in the field as well as general use does require

some considerations. The curved nature of objects (i.e. glass jar, stomach pill, animal) may result in incomplete and less accurate temperature imaging of the entire surface. For example, Ash et al. (1987) demonstrated with an isothermic balloon that peripheral surface temperatures were 2 – 4 °C cooler than the perpendicular surface temperature from the camera lens to the object. Ash et al. (1987) similarly concluded that thermography is a very valuable tool, but should be used to look at patterns of heat loss and not be used for absolute temperatures. Efforts to minimize the error of environmental (non-instrument) parameters are advantageous to the user to obtain consistent and comparable thermal results with this technique (Ohman, 1981). During field studies, this means collecting data during similar weather conditions and at consistent times of the day to control for ambient air temperature, relative humidity, and solar radiation due to cloud cover. This would be optimal for comparing physiological state of individuals (i.e., resting, foraging). While it was shown in Table 2.2 that the required parameters collected for the software have very minimal influence at this emissivity range, in non-field settings, without the advantage of a controlled setting, these parameters will be useful in order to compare individuals if non-longitudinal data on individuals are unrealistic. In captive settings, distance can be set so that the object fills the frame of view in most situations. In field settings this may not be possible. Increased distance to the target in a non-controlled setting will be impacted by environmental factors, particularly wind velocity (Moen and Jacobsen, 1974), as it creates noise in the resultant image quality.

Biological parameters

Blubber storage depots and tissue uniformity vary between harbor seals and Steller sea lions and most likely affect temporal variation seen in regional skin surface temperature. Harbor seal blubber is typically homogenous in both depth and composition, whereas Steller sea lion blubber includes interstitial tissue and varies in depth along the body (Mellish et al., 2007; Rosen and Renouf, 1997). Long-scale (i.e., seasonal) temporal skin temperature variations in regions were detected in both species, but were more prominent in harbor seals. Mauck et al. (2003) specifically looked at discrete areas visible via IRT however, no spatially or temporally consistent thermal windows were observed for up to 2 hours within an individual session. While our study concentrated on the presence of consistent regions within 5 – 10 minutes of hauling out and across one annual cycle, these regions were later defined as hot or cold spots and then further examined to determine which of these spots may actually be thermal windows. This allowed us to eliminate the potentially confounding variable of wet versus dry pelage as well as to identify areas where animals might be actively losing heat at the critical thermoregulatory transition from water to air. As stated above, we observed spatially consistent regions as well as temporal consistencies amongst seasons in both harbor seals and Steller sea lions. The higher the air temperature, the more likely evaporation will play a key role in the number of regions showing up as heat dissipaters, but may not actually be areas that can both truly dissipate and retain heat. The mean surface temperature of these spatially recognized windows will also vary with air temperature. In both species, variations were most notable pre and post winter, but minimal variation was noted between the

reproductive and molt periods. During reproduction and molt, seals and sea lions haul out of the water to facilitate pupping and promote circulation for new hair growth which requires extra expended energy. Higher surface temperatures were also noted between the reproductive and molt seasons and lowest during the winter. These temporal variations are to be expected from an animal transitioning from a water medium where temperatures stay relatively stable annually to an air environment that has notable seasonal temperature flux. In addition, insulative properties (i.e., blubber depth) also fluctuate similarly, thickest in the winter decreasing from reproductive to molt and then increasing again during winter. Lastly, no effects due to water temperature even after a relatively short time period after immersion were found. Two problems arise here, as there was a seasonal effect in water temperature and Figure 2.6 suggests that there was an annual trend in hind flipper temperature and water temperature. There does seem to be a trend of low variance in water temperature with high variance in skin surface temperature, which may be why there was no correlation. Even under stable conditions, blood flow to the surface can be highly variable depending on the immediate needs of the animal. Thus, the flipper may look cooler through IRT in relation to other regions of the body, but this does not mean that the flippers are necessarily cold.

In the present study, animals were imaged within one physiological state (i.e., resting) to minimize within and between regional variation due to thermal state and to ultimately determine what areas act as thermal windows in rested harbor seals and Steller sea lions. Several studies suggest that the main trunk of the body is not a 'normal' thermal window.

Instead, the extremities will demonstrate the most day to day temperature fluctuation with changes in ambient air temperature (Cuyler et al., 1992; Choi et al., 1997). Whole body, torso and head each include opposing insulated and vascularized spots and therefore are not considered thermal windows. Stewart et al. (2008) found that eye temperature decreases with pain, such that it may be a potential reference to physiologic and/or metabolic state of the animal. This site may be more closely related to internal core temperature as it is the only region with no insulative properties and therefore also not considered a 'normal' thermal window. The highly vascularized muzzle region is most likely a hot spot as this area supports a sensitive sensory tactile system (Dehnhardt et al., 1998). The hip region often presents itself as a hot spot which is most likely related to blubber thickness (Mellish et al., 2007). Therefore, shoulder, axillae, and both fore and hind flippers may be regions that could be suggested as thermal windows. Under the conditions of this study, the flippers consistently showed up as cool spots, however during several excluded sessions over the year within the sea lions, it was easily identifiable when an individual had been actively foraging or swimming as both fore and hind flippers showed vascularized areas that were hot. Although regional variability was slightly different between the two species, it was to be expected due to complications of sample size variation, morphology, and age differences.

Shortly after immersion, surface temperature tends to equilibrate to temperature of the water medium (Hayward and Keatinge, 1981). Depending upon weather conditions, upon emersion the skin surface will begin to show patterns that suggest areas of heat

dissipation and areas of heat retention. Whether these patterns are independent or as a combination of variables such as superficial blood vessels, insulation variations, or as a result of physical processes of heat transfer is still to be determined. Early physiological studies that looked at skin surface temperature found the extremities to be an area of variable heat dissipation while the torso of the body remains an area of stability due variable insulator gradients in the two areas (Irving and Hart, 1957; Irving et al., 1962). The high correlation between whole body surface temperature and torso surface temperature in both species does suggest constancy between the two regions while at rest. It is often assumed that an animal with a lower level of insulation will produce more internal heat (displaying higher surface temperature). This may result in a higher overall metabolism to maintain body temperature, which in turn would lead to lower body condition than an individual with a higher level of insulation in the same ambient conditions. Future study of the relationship between blubber depth and surface temperature would allow for a more precise interpretation of surface heat patterns and body condition in these species (i.e., anatomy versus physiological state).

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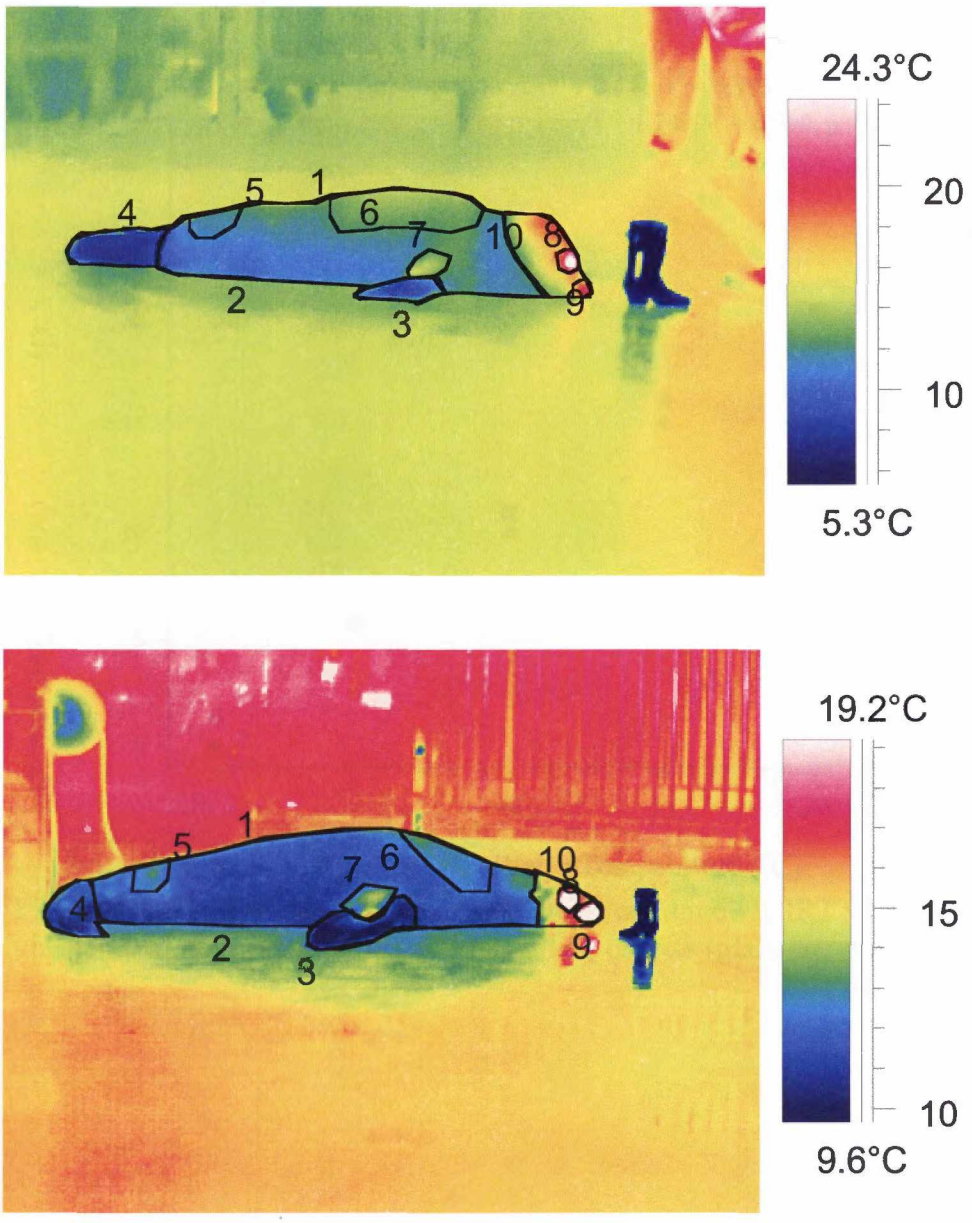

Figure 2.1. Thermal image depiction of patterns measured through infrared thermography on A) a juvenile female harbor seal *(Phoca vitulina)*, and B) an adult female Steller sea lion (*Eumetopias jubatus*). 1 – whole body, 2 –torso, 3 – fore flipper, 4 – hind flipper, 5 – hip, 6 – shoulder, 7 – axillae, 8 – eye, 9 – muzzle, 10 – head.

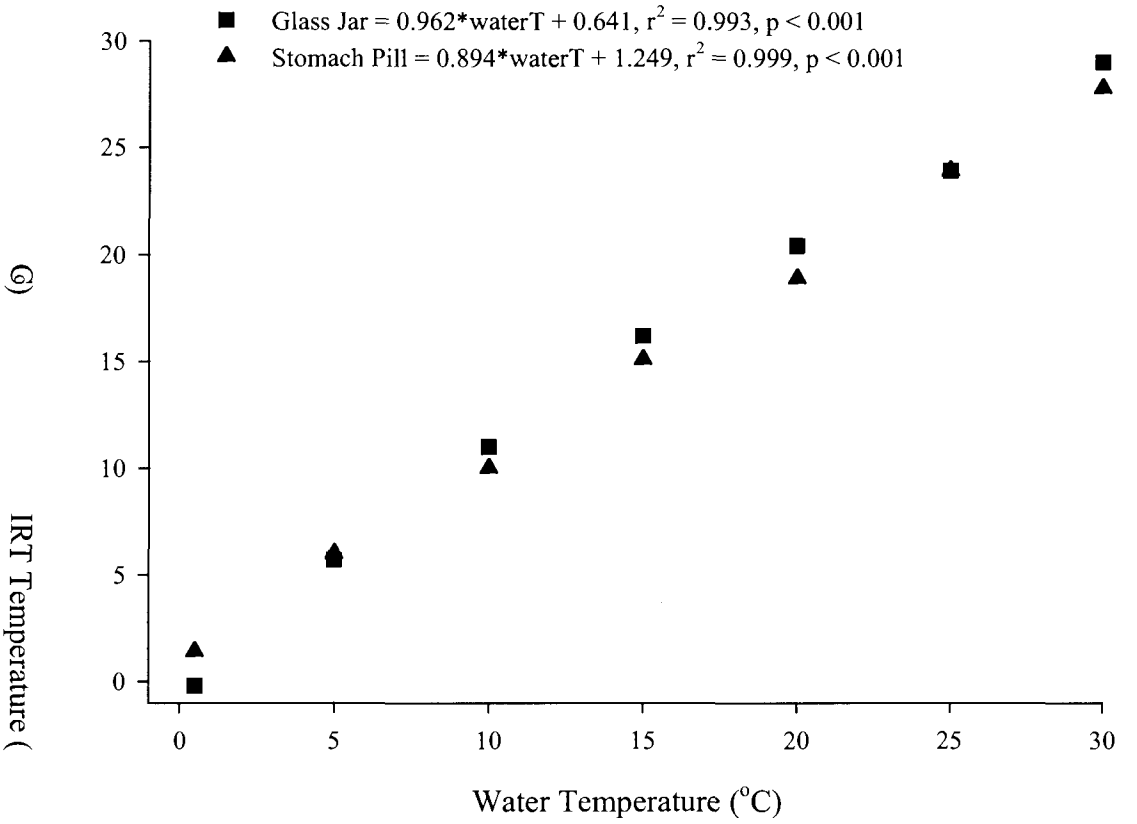

Figure 2.2. Linear regression of temperature measured via IRT vs. temperature measured via standard mercury thermometer using two objects (glass jar, stomach pill).

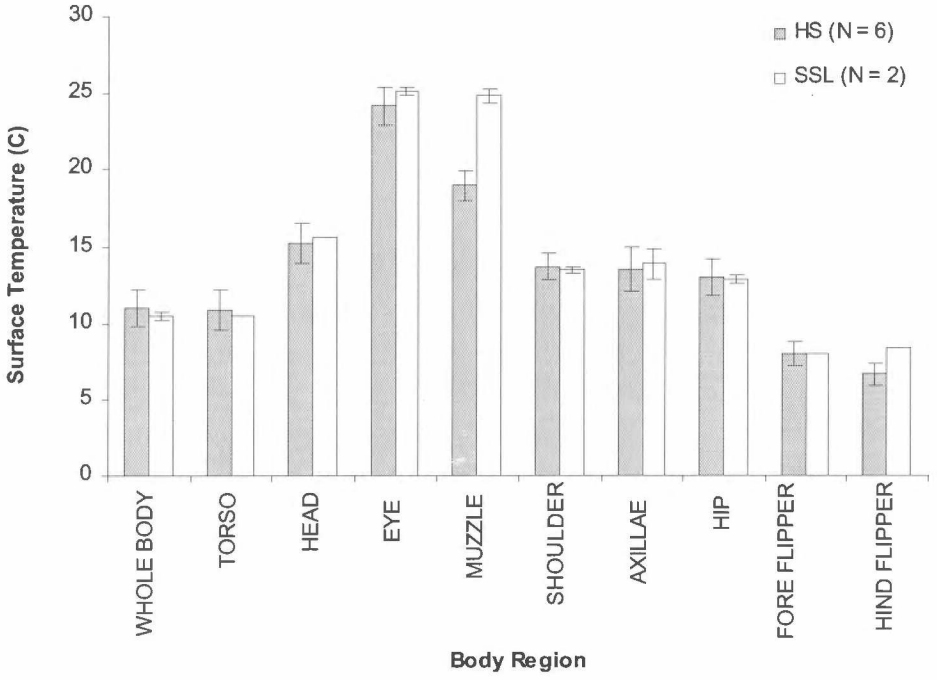

Figure 2.3. Regional annual surface temperatures in harbor seals (HS) (*Phoca vitulina*) and Steller sea lions (SSL) (*Eumetopias jubatus*) as measured by infrared thermography. Whole body, torso and head regions are annual means of average surface temperature± SD; head, eye, muzzle, shoulder, axillae and hip regions are annual means of maximum surface temperatures; fore and hind flippers are annual means of minimum surface temperatures. SD is not visible in some regions for SSL due to no difference between individual means.

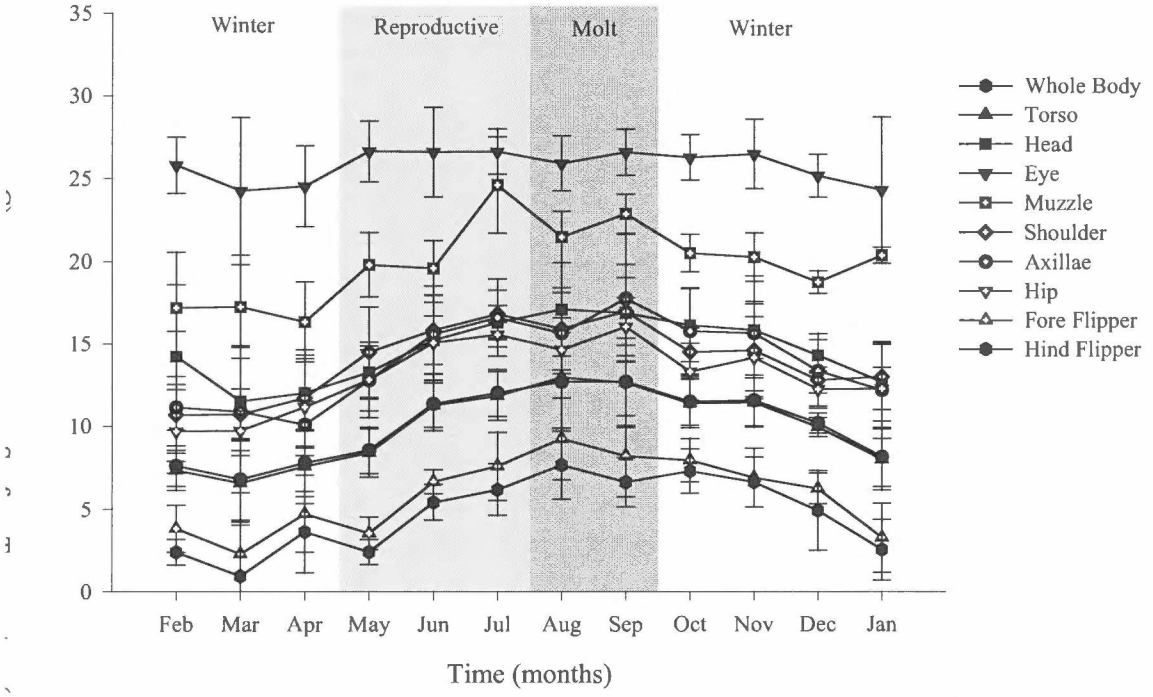

Figure 2.4. Average skin surface temperatures by month and season for harbor seals, *Phoca vitulina,* (n = 6) measured by infrared thermography. Whole body, torso and head regions are averages of individual animal mean monthly values of surface temperature (^{o}C) ± SD; head, eye, muzzle, shoulder, axillae and hip regions are averages of individual animal maximum monthly values of surface temperatures; fore and hind flippers are averages of individual animal minimum monthly values of surface temperatures.

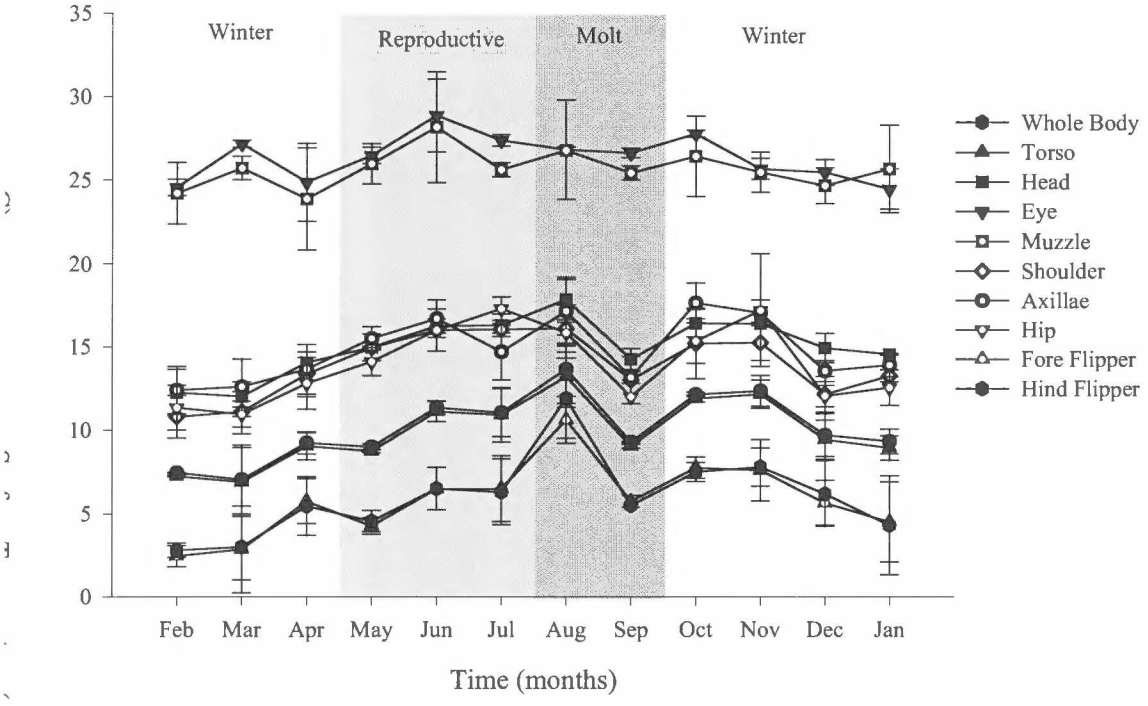

Figure 2.5. Average surface temperatures by month and season for Steller sea lions, *Eumetopias jubatus,* (n = 2) taken through infrared thermography. Whole body, torso and head regions are averages of individual animal mean monthly values of surface temperature (°C) ± SD; head, eye, muzzle, shoulder, axillae and hip regions are averages of individual animal maximum monthly values of surface temperatures; fore and hind flippers are averages of individual animal minimum monthly values of surface temperatures.

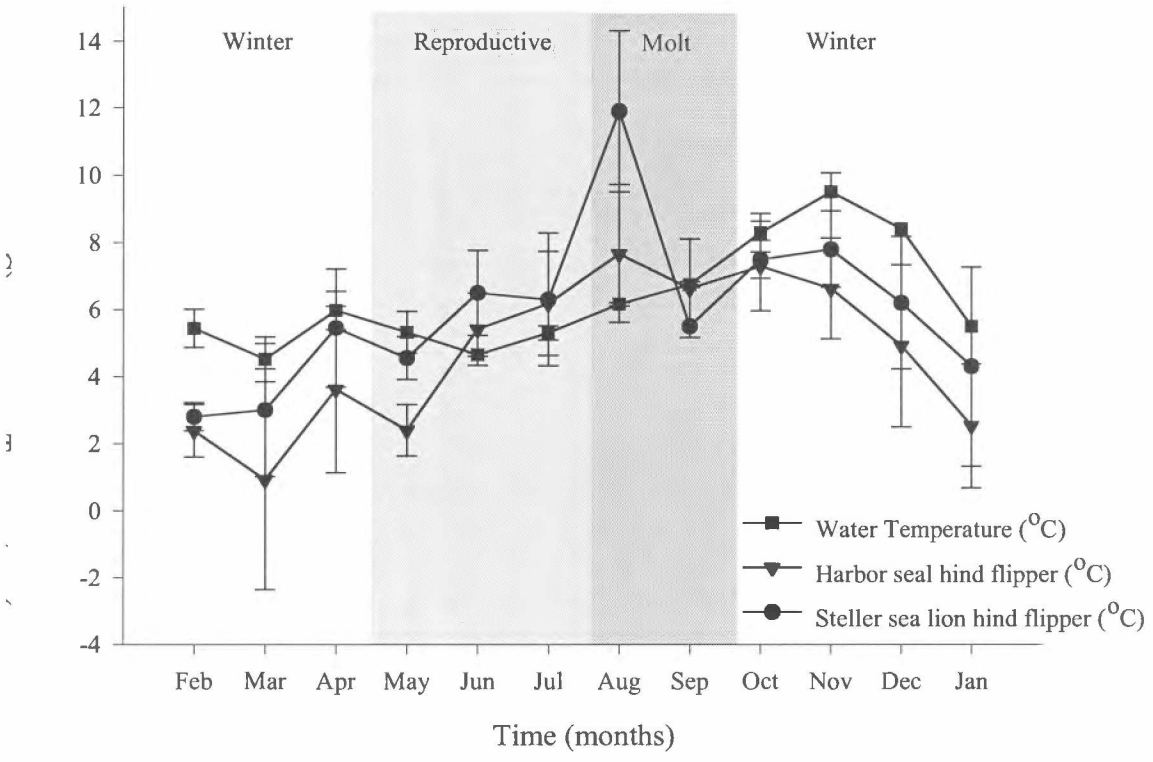

Figure 2.6. Average monthly and seasonal sea water temperatures and minimum skin surface temperatures of the hind flipper regions of harbor seals (n = 6) and Steller sea lions (n = 2). All temperatures are in °C and means are shown with ± SD.

Table 2.1. Number of image sets collected via infrared thermography for six juvenile female harbor seals (*Phoca vitulina*) and two adult female Steller sea lions (*Eumetopias jubatus*) during project duration, February 2007 – January 2008. Date in superscript following ID name is birth year of individual.

	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Total
Seals													
PVAT^{2004}	2	1	2	3	2	3	1	2	2	3	2	2	25
PVQI^{2004}	2	2	2	2	2	4	1	2	3	2	2	2	26
PVSH^{2005}	2	2	1	2	2	2	2	2	2	3	2	3	25
PVSI^{2005}	1	3	1	2	3	2	2	2	2	2	2	3	25
PVSU^{2004}	2	2	2	3	2	3	1	2	2	3	2	2	26
PVTI^{2005}	2	2	1	2	3	2	1	3	2	2	2	2	24
Sea Lions													
EJKI^{1993}	3	2	2	5	4	3	1	2	3	2	2	2	31
EJSU^{1993}	4	3	2	5	2	4	1	2	3	2	2	2	32

Table 2.2. Effects of parameter modification on software calculation of whole body surface temperature in a harbor seal (PVQI) and Steller sea lion (EJSU) image session. Relative humidity, distance, ambient air temperature, and reflected temperature were modified by an increase and decrease of 10% from its original value, while emissivity was modified by 0.02 points. These values were changed one at a time while holding all other parameters constant (i.e. emissivity was changed from 0.98 to 0.96, all other parameters were held at the standard (std)). The table indicates the modified temperature for each modification (noted by species and parameter). Original temperature output with actual parameter measurements for PVQI was 8.9 °C and for EJSU was 12.1 °C.

Software Parameters	PVQI Std	(-) 10% Change	(+) 10% Change	EJSU Std	(-) 10% Change	(+) 10% Change
Emissivity	0.98	8.9	9.0	0.98	12.0	12.2
Relative Humidity (%)	62.6	8.9	8.9	62.9	12.1	12.1
Distance (m)	3.0	8.9	8.9	7.6	12.1	12.1
Ambient Air T (°C)	19.6	9.0	8.9	19.7	12.2	12.1
Reflected T (°C)	10.6	9.0	8.9	15.6	12.1	12.1

Table 2.3. Mean coefficients of variation (COV) of each region for harbor seals (*Phoca vitulina*) and Steller sea lions (*Eumetopias jubatus*) during the study period February 2007 – January 2008. COVs were calculated for each region (from monthly average temperatures for whole body, torso and head; maximum temperatures for eye, muzzle, shoulder, axillae and hip; minimum temperatures for fore and hind flipper) per individual then averaged for one value per region across animals.

Region	Harbor Seal (n = 6)	Steller sea lion (n = 2)
	Coefficient of Variation (COV)	Coefficient of Variation (COV)
Whole Body	6.8	4.5
Torso	6.9	2.9
Head	10.4	8.3
Eye	11.8	4.8
Muzzle	11.9	6.0
Shoulder	12.3	3.8
Axillae	8.4	3.2
Hip	11.2	4.6
Fore Flipper	6.8	3.5
Hind Flipper	5.8	2.7

Table 2.4. Manual rank of mean surface temperatures in the harbor seals (*Phoca vitulina*) and the Steller sea lions (*Eumetopias jubatus*). Rank is based on 1 being the hottest through 10 being the coolest. Multiple runs of Mann-Whitney tests to identify regions that significantly change between seasons are represented in the line between season columns. Significance between winter to reproductive, reproductive to molt and molt to winter is as follows: (——), $p \leq 0.001$; (– – –), $p \leq 0.01$; (.....), $p \leq 0.05$.

Harbor seals

(n = 6)

Region	Winter Rank		Reproductive Rank		Molt Rank	
Whole body	7	– – –	7		7	——
Torso	8	– – –	8		8	——
Head	3	.....	3		3	——
Eye	1		1		1	
Muzzle	2		2	.....	2	——
Shoulder	5	——	4		5	——
Axillae	4	– – –	6		4	——
Hip	6	——	5		6	——
Fore Flipper	9	– – –	9		9	——
Hind Flipper	10		10		10	——

Steller sea lions

(n = 2)

Region	Winter Rank		Reproductive Rank		Molt Rank	
Whole body	7		8		7	
Torso	8	.....	7		8	
Head	3		3		3	
Eye	2		1		1	
Muzzle	1		2		2	
Shoulder	5	.....	4		5	.....
Axillae	4		6		4	
Hip	6		5		6	
Fore Flipper	10	.....	10		10	
Hind Flipper	9	.....	9		9	

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CHAPTER 3[2]: THE THICK AND THIN OF BODY CONDITION: DOES BLUBBER DEPTH INFLUENCE SKIN SURFACE TEMPERATURE IN PINNIPEDS?

Infrared thermography (IRT) was assessed as an alternate tool to evaluate body condition in long-term captive, juvenile female harbor seals, *Phoca vitulina*, (n = 6) and adult female Steller sea lions, *Eumetopias jubatus*, (n = 2) through the comparison of skin surface temperature to blubber depth as measured by ultrasonography. Eight site-specific locations were measured using a FLIR P25 infrared camera and a SonoSite Vet180 portable imaging ultrasound two times per month from February 2007 – February 2008. It was found that the most variable skin surface temperature site in harbor seals was the least variable and thinnest blubber location annually (D2). While inversely the least variable skin surface temperature site was the most variable blubber depth and thickest site annually (L2). One of the two Steller sea lions showed a similar trend with the least variable skin surface temperature site being the most variable blubber depth site (D5). Seasonal change was apparent in IRT and ultrasound measures in both species, having overall increases in surface temperature from winter to summer with concurrent decreases in blubber depth. While some site-specific significant relationships were found between skin surface temperature and blubber thickness, insulation level alone explained a very small portion of the variance.

Key words: Harbor seal, *Phoca vitulina*, Steller sea lion, *Eumetopias jubatus*, ultrasound, infrared thermography, body condition, pinnipeds

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INTRODUCTION

Body condition in pinnipeds directly impacts thermoregulation as blubber serves the dual purpose of chief insulatory layer and primary energy depot (Ryg et al., 1988) in most species. While assumed to be critical for heat retention by animals in cold waters, excess blubber in warm conditions can result in a thermoregulatory challenge. Ultimately, blubber serves as a source of multiple thermal constraints that affect overall energy balance (Rosen et al. 2007). Buoyancy in water and therefore locomotive and foraging capabilities are also impacted by total body fat (Beck et al., 2000; Koopman et al., 2002). Blubber depth is not static, as changes in body condition can occur due to variation in prey availability, seasonality of energetic requirements, or physiological demands due to different life-history stages.

Seasonal variation in pinniped body condition, as measured by blubber thickness, has been well-documented in many species (grey seals, Boyd, 1984; Sparling et al., 2006; harbor seals, Pitcher, 1986; Mellish et al., 2007; harp seals, Nilssen et al., 2001; hooded seals, Thordarson et al., 2007; ringed seals, Ryg et al., 1990; Steller sea lions, Mellish et al., 2007), which is important for the accurate development of energetic models and detailed understanding of life history parameters. Common to all studies was the finding of a maximum blubber depth during the winter that decreased during the reproductive and molt periods. Body condition has been determined through various methods and levels of invasiveness from culled animals (Boyd, 1984; Pitcher, 1986; Thordarson et al., 2007), non-fatal but invasive collections (isotope dilution, Slip et al., 1992; Beck et al., 2000; Rutishauser et al., 2004), and completely non-invasive methods (morphometrics,

McLaren, 1958; ultrasound, Gales and Burton, 1987; Slip et al., 1992; Mellish et al., 2004, 2007). Each method provides a different level of accuracy that must be appropriate to the research and logistical needs of the study. In all cases, however, some measure of animal handling or contact has been required.

With advances in technology, non-invasive methods of conducting research are becoming more popular as more stringent permit restrictions are placed on populations of marine mammals at risk. Infrared thermography (IRT) is a technique that has mostly been used for diagnostic purposes in veterinary and wildlife applications with high potential for future studies (see review, McCafferty 2007). As a completely non-invasive method that requires no physical contact with the animal, it allows the operator to view heat emission from an object either as a captured still image or in real-time video. Specifically, it provides estimates of surface temperature, which with proper validation may provide an estimate of body condition, as well as indication of site-specific thermoregulatory capabilities, physiological state, and evidence of inflammation, parasitism or wound healing.

In this study, we directly assessed the surface temperature of two pinniped species in relation to concurrent measures of body condition through the use of ultrasonography. Our specific objectives were to:

1. Compare location-specific surface temperature measured with IRT to blubber depth measurements obtained at eight body sites in harbor seals (*Phoca vitulina*) and Steller sea lions (*Eumetopias jubatus*).
2. Assess applicability of IRT to derive proxy measures of body condition.

MATERIALS AND METHODS

Captive animals (harbor seals, Steller sea lions).—Six female juvenile harbor seals (*Phoca vitulina)* and two adult female Steller sea lions (*Eumetopias jubatus*) housed at the Alaska SeaLife Center (ASLC) in Seward, AK were the subjects of a larger year-long study of the feasibility of IRT to identify consistent thermal windows in pinnipeds (Chapter 2: Thermal windows in seals and sea lions: what's hot and what's not?), with a further assessment of the data as a proxy for the measurement of body condition. IRT data were compared to ultrasonography, a commonly used technique to measure blubber thickness (Mellish et al., 2004). All juvenile harbor seals were captured from the Prince William Sound and eastern Kenai Peninsula, AK areas as newly weaned pups (PVAT, PVQI, PVSU 2004; PVSH, PVSI, PVTI 2005). The two adult female Steller sea lions (EJKI, EJSU), were captured and brought into captivity as pups in 1993 from Maggott Island, Canada.

Due to other ongoing research projects, the juvenile harbor seals were on a mixed diet of pollock (*Theragra chalcogramma*), capelin (*Mallotus villosus*), squid (*Loligo opalescens*) and a lipid regulated diet of either high fat Pacific herring (*Clupea pallasii*; PVAT, PVQI, and PVSI) or low fat Atlantic herring diet (*Clupea harengus*; PVSH, PVSU, and PVTI) (L. Polasek, unpublished data). Diets of the two Steller sea lions consisted of pollock and Pacific herring supplemented with pink salmon (*Oncorhyncus gorbuscha*) three days a week. All diets included a mass-appropriate dose of a multivitamin (Mazuri 5# Marine Mammal Tablet).

Data collection and equipment use.—Images were collected two times a month from February 2007 through January 2008. Two investigators collected thermal images during the year-long study to reduce variability in data due to operator technique. A FLIR P25 infrared camera (FLIR Systems, Danderyd, Sweden) was used to take the thermograms. The camera has a thermal temperature sensitivity of <0.10 at 30 °C and factory calibrated to an absolute accuracy of ± 2 °C. Relative humidity (%) and ambient air temperature (°C) were measured with a Sper Scientific Ltd #850070 Mini Environmental Quality Meter (Sper Scientific Ltd, Scottsdale, Arizona, USA) with an accuracy of ± 6% for relative humidity and ± 1.2 °C for air temperatures, both measured to the nearest 1/10 of a percentage point or 1/10 of a degree.

Images were taken indoors under artificial fluorescent lighting or under naturally lit conditions of an outdoor exhibit, dependent upon the husbandry and research requirements of the captive animals. A plain concrete flooring, a concrete flooring covered with an epoxy coated paint or an aluminum surfacing was the substrate for all thermal images. A session typically involved the transfer of an animal from a holding area to the room where images took place (approximately 15 meters). For consistency, only wet animals were imaged, within 5 – 10 minutes of hauling out. The thermographer stood in a fixed position while the animals were behaviorally controlled to either remain stationary or move between images. Up to eight images were taken per animal per session. Thermograms were obtained at an approximate angle of 90 degrees between camera lens and right lateral side of the animal. An image collected at a 180 degree turn away from the animal was used as the reference temperature. Distance was set so that the

animal would fill the frame of the picture from tip of tail to tip of nose. For ease of focus and increased contrast, a gray color palette was chosen for image collection. Session time lasted approximately 10 minutes per animal. Anesthesia, sedation and/or restraint were not used.

In conjunction with thermal imaging sessions, blubber depth was measured with a portable imaging ultrasound, SonoSite Vet180 with a C60/5-2 MHz broadband transducer (Sonosite, Inc., Bothell, Washington, USA). Following methodology as explained in Mellish et al. (2007) ultrasound data were collected by one of two investigators on the right side of the animal throughout the study period. Four lateral and dorsal sites (Figure 3.1) were used on both species to measure skin plus blubber depth and later IRT surface temperature captured from the image. Body mass was taken of each individual imaged immediately pre- or post-session by husbandry staff (Transcell, Model TI-500-SL, Accurate Scales, Terre Haute, IN, USA)

Data Analysis.—To more accurately compare ultrasonography measurements to appropriate locations on the corresponding thermal image, measurement points of blubber depth taken via ultrasound were reduced to relative size on the image (i.e. contact surface area of the ultrasound transducer on the actual animal was scaled down to relative size on the animal in the IRT image).

All images were analyzed using FLIR ThermaCam Researcher Pro 2.8 SR-1 (FLIR Systems, Danderyd, Sweden). The standard palette chosen for medical thermography, the rainbow color palette, was chosen for image analysis. The software corrected for the following parameters: emissivity, distance, reflected temperature,

ambient temperature, and relative humidity. Image sets from one individual of each species (PVQI and EJSU) were chosen at random (August 22, 2007) to compare surface temperature patterns among the eight image angles. With the exception of biopsy and/or injection sites, thermal patterns were equally visible amongst all positions. Due to the symmetry of thermal patterns, the right lateral image was chosen for analysis and statistical purposes. All other images were used as references only.

An analysis of IRT by seasons [defined as winter (Oct – Apr), reproductive (May – Jul) and molt (Aug – Sep)] revealed no significant seasonal changes between regional skin surface temperature in the reproductive and molt seasons with the exception of the muzzle in harbor seals (J. Nienaber, unpublished data). Therefore, temperature data were grouped into two seasons for both species, defined as winter (Oct – Apr) and summer (May – Sep). To assure that each season and each individual was equally represented, nine consecutive data points were chosen for each season and individual that was neither at the beginning or end of the season.

SYSTAT 10 (SYSTAT Inc., Evanston, IL, USA) was used for statistical analysis. All data are represented with mean ± standard deviation (SD) and p-values of ≤ 0.05 were considered significant. Seasonal variability and changes in body mass were assessed with multiple runs of the non-parametric Mann-Whitney U-test using seasons and data described as above. An annual coefficient of variation (COV) was used to describe variability across sites, mean COV for each species at each site was represented. Data were further reduced to one data point per season per animal and simple linear

regressions were used to describe the site-specific relationships between ultrasound and IRT temperature sites.

RESULTS

Changes in body mass.—Harbor seal body mass averaged 32.2 ± 6.5 kg, with a significant seasonal effect ($U = 2012.5$, $p < 0.001$). Seals were lightest during summer (30.1 ± 6.5 kg) and heaviest in winter (34.3 ± 6.5 kg). Sea lion body mass did not vary over the year (214.8 ± 5.5 kg).

Changes in skin surface temperature estimated via IRT.—Seasonal skin surface temperatures differed significantly at all sites in harbor seals (Table 3.1), with the lowest temperatures in winter (L5, 7.6 ± 0.7 °C) and highest during summer (D2, 12.8 ± 1.7 °C). The annual mean of the average temperatures of all eight measurement sites combined was 9.8 ± 0.6 °C and annual mean of torso temperatures was 10.0 ± 1.0 °C. Steller sea lion skin surface temperature differed significantly between seasons at all sites (Table 3.2). Lowest temperatures were seen in winter at L3 and L4 (7.4 ± 0.8 °C at both sites), while highest surface temperatures were noted in the summer (D5, 11.1 ± 1.0 °C). All eight sites had an annual mean of average temperatures from all sites of 9.1 ± 0.5 °C and annual torso temperature was 9.4 ± 0.7 °C. All sites in both species increased in temperature from winter to summer.

Changes in blubber depth measured via ultrasound.—Mean blubber depth also varied significantly between seasons at all sites in harbor seals (Table 3.3), with a minimum mean blubber depth at site D2 during the summer (1.3 ± 0.2 cm) and maximum

mean blubber depth at site L2 during the winter (3.0 ± 0.4 cm). In contrast, only two of the eight sites had a significant seasonal change in the sea lions (L2 and L5, Table 3.4).

Variability between sites.—Using COV to determine variability between sites (Table 3.1), it was found that in four of the six harbor seals, the least variable annual skin surface temperature was found at site L2 (10.32) while the most variable site was D2 (12.34, Table 3.1). Conversely, blubber depth at site D2 was the least variable site annually (0.05), while site L2 was the most variable site (0.29, Table 3.2). In the Steller sea lions, L3 exhibited the least variable skin surface temperature (5.28, Table 3.3), while L5 was most variable (5.73). Site D2 was the least variable in blubber depth (0.06, Table 3.4), while site L2 was the most variable (0.27).

Relationship between techniques.—Visual representations between site-specific locations of skin surface temperature measured via IRT and of blubber thickness measured via ultrasonography are shown in Figure 3.2. Annual site-specific relationships between skin surface temperature and blubber depth were significantly related at all sites in harbor seals except for D3 and L2 (simple linear regression, $p \leq 0.05$, Table 3.5). However, in the sites that are significant, the proportion of the variance seen in skin surface temperature that was explained by blubber thickness was small, except for site L5 (r^2 =0.69, all other sites $r^2 \leq 0.50$). In Steller sea lions, only one site-specific location was significantly related in skin surface temperature and blubber thickness annually (D4, $p = 0.004$, $r^2 = 0.99$, Table 3.5). When IRT and ultrasound findings were visually assessed by individual in the harbor seal, the seals showed a slight trend in all sites, except for L5. An inverse trend between skin surface temperature and blubber thickness, suggested an

approximate 2.5 cm blubber depth threshold, where beyond 2.5 cm skin surface temperature no longer inversely tracks blubber depth (Figure 3.3).

DISCUSSION

Relationship of skin surface temperature to blubber thickness.—Understanding multiple methods to assess animal condition and data collection can prove useful when logistical complications such as permit or species restrictions and location inaccessibility arises. IRT is a useful non-invasive method that has been shown to prove functional in both captive and wildlife populations (early diagnostics, Spire et al., 1999; Eddy, 2001; Turner, 2001; Xie et al., 2004; Dunbar and MacCarthy, 2006; physiological studies, Lancaster et al., 1997; Williams et al., 1999; Simon et al., 2006; assessment of thermal windows, Mauck et al., 2003; Willis et al., 2005).

In our comparison of non-invasive techniques, there was no simple relationship between skin surface temperature (IRT) and blubber depth (ultrasound). The proportion of variance that was explained by blubber depth was variable along the body (i.e. Table 3.5), suggesting multiple physiological parameters can have an influence on skin surface temperature. The expected underlying effect of increased insulation paired with reduced skin surface temperature was apparent (Figure 3.2), however, high variance and low sample size may have precluded significance. If within-site variance can be controlled by determination of the factor causing the inconsistency (i.e. individual metabolic rate, ambient air temperature, physiologic state), then there may still be a possibility that

blubber thickness can be predicted by skin surface temperature (i.e., as part of a more complex model).

A similar and interesting trend found within the harbor seals, was that the least variable IRT site (L2) was also the most variable site in blubber depth. Likewise, the most variable IRT site (D2) was also the least variable site in blubber depth. Both of these sites were identified as potential thermal windows in Chapter 2, suggesting a site-specific causal, anatomical relationship between thermoregulatory layer (i.e., blubber) and surface heat via IRT; however, this relationship may be again modulated by several confounding physiological parameters (i.e., metabolic rate). The trend in IRT and ultrasound findings assessed on the harbor seals reveals a potential inverse relationship with a threshold at 2.5 cm blubber depth. Beyond 2.5 cm blubber depth temperature no longer tracks blubber thickness (Figure 3.3). The high variability between animals masks this relationship when the data are pooled (Figure 3.2). Blubber depth at L5 was similar to that at D5, but D5 inversely tracked temperature and L5 did not. The discrepancy in these two sites was most likely due to anatomical and physiological site-specific characteristics. In the Steller sea lion, D5, an area close to the caudal gluteal vein, lies parallel to the spine and therefore may have a relatively higher surface temperature within this area. L5 lies above bony anatomical features. These two sites exemplify the importance of understanding thermal windows as they relate to morphologic structure. Furthermore, L2, the site with most variable blubber depth and most stable skin surface temperature, may be an indicator for body condition. If L2 measures above the 2.5 cm blubber threshold depth, skin temperature will likely display little to no variation.

Whereas, a site like D2, that has the most variable skin surface temperature yet most stable blubber depth, may play a more active role in heat dissipation along the body. Similarly in sea lions, temperature inversely tracks changes in blubber depth with the exception of L5, but did not show a maximum threshold effect in blubber depth.

Seasonal effect using skin surface temperature.— Limited studies exist that assess seasonal effects on skin surface temperatures through IRT, although one study compared ambient temperature to skin temperature in humans (Livingstone et al., 1987). While core temperature was maintained, individuals with higher body fat content exhibited cooler overall surface temperature. That study found that skin temperature differences were greater over a range of ambient air temperatures in individuals that had less fat than individuals that had more fat. Harbor seals had a high annual variability in blubber thickness and lower overall blubber thickness relative to sea lions. This may account for the greater variation seen in skin surface temperature, and thus may potentially correlate with ambient air temperature.

Seasonal effect using blubber thickness.—In an earlier study of seasonal and species-specific variation in blubber depth, Mellish et al. (2007) focused on adult harbor seals and Steller sea lions (same individuals as current study). As with Mellish et al. (2007), harbor seals in the current study were heaviest in the winter, site L2 was the thickest and most variable blubber depth site overall, while site D2 was also found to be the thinnest site. Steller sea lions during this study also showed no seasonal effect in blubber depth as in the previous study (Mellish et al., 2007) and site L2 was also found to be the most variable. Wild, culled harbor seals display a parallel heaviest mass in the

winter, with decreased blubber thickness from reproductive to molt seasons (Pitcher, 1986).

Life history implications.—Adult phocids, including harbor seals, have a relatively long period of fasting that includes being hauled out for variable lengths of time, relatively short weaning time, different morphology being more rotund than the Steller sea lion, and equipped with a hind flipper rotor action while in the water (Riedman, 1990). Otariids, including Steller sea lions on the other hand, have relatively short periods of fasting, have weaning time between one - two years, are built more elongated than spherical, and use their front flippers for movement in the water medium (Riedman, 1990). While this study cannot directly compare both species as they are at different ages in reproductive maturity, significant seasonal changes in skin surface temperature in both species signifies that multiple parameters are the cause of the variance observed (i.e., blubber thickness, ambient air temperature, physiological or metabolic state, etc.). Summertime is a high energetic time as both species are pupping and sequentially hauled out and fasting during this time. During these fasting periods, mothers with pups rely on winter fat stores for energy utilization, decreasing energy in its depots throughout the summer months and having an annual low that occurs during the molt prior to winter. This will have a significant effect in skin surface temperature as the temperature gradient will be lessened between individual core and the peripheral skin surface. Having a lower temperature gradient (i.e., core to skin surface, skin surface to ambient air) at a time when the animal spends the majority of its time on land, during the warmest months of the year, reduces the rate of heat lost from the individual (Scholander

et al., 1950). Having a low core to skin surface gradient, due to low insulation and a low skin surface to ambient temperature gradient, would reduce overall heat flux thus the rate at which overall energy expended may be reduced. In other words, at high ambient temperatures, the difference between core temperature and skin surface temperature is minimized (Hilsberg-Merz, 2008). This would be thermally advantageous as it increases the amount of time an individual could be hauled out while minimizing foraging requirements due to low energy expenditure.

Physiological state will also have an impact on site-specific heat patterns as areas that are known as thermal windows (i.e., active areas of heat emission and/or retention) will vary between a rested animal *vs.* an animal that is under physical exertion. Areas that are less insulated may also show increased heat patterns (i.e., hip/D5), but species-specific validation is necessary to distinguish low insulation *vs.* thermal windows (i.e., shoulder/D2, axillae /L2, flippers) that actively dissipate heat (Whittow et al., 1975; Clark et al., 1977; Pabst et al., 2002; Simon et al., 2006; Hilsberg-Merz, 2008). These areas will also be able to actively conserve or dissipate heat depending upon the thermal gradient that exists between skin surface temperature and the environmental temperature (Carpenter, 1986).

Technique applications and future directions.—The ability of skin surface temperature to predict blubber thickness may not be suitable for pinnipeds until further studies can explain the variance observed in the data. However, the ability to detect small changes in skin surface temperature in pinnipeds allows us to visually see skin surface temperature at a non-invasive level as well as diagnoses of ailing individuals (i.e.,

soft tissue inflammation, fore flipper blisters, skin surface bruising, and bite wounds). Often IRT is used to diagnose soft tissue injuries that immediately do not present symptoms and go unnoticed during physical examination (i.e., lameness in equine) or for animals that are hard to maneuver or one-on-one contact is impossible (i.e., toe injuries in elephants and horn infections in rhinoceros; Hilsberg-Merz, 2008). Pregnancy has also been validated for several species in captivity (i.e. elephants, rhinoceros, giraffes) with two conditions met prior to using thermography: sufficient size of the fetus for heat production to become visible and an optimal temperature range of 15 – 18 °C to allow for excess heat to be conducted through the body (Hilsberg-Merz, 2008). An adult pregnant female harbor seal (PVCH) not included in the current study was opportunistically imaged. The lateral position of the pup was imaged using IRT during one image session in late pregnancy (3 months prior to birth). Ambient temperature was 14 °C and while this image was the only image seen with fetus, ambient temperatures of other images were kept in a fairly set range and validation of pregnancy detection through skin surface temperature via IRT should be further looked into for pinnipeds. Consistent conditions at each session are also an advantage if trying to qualitatively compare individuals, including wetness of the animal and physiological state. Future studies should include the use of individuals in an exercised physiological state *vs.* a rested state to define the impact of energy expenditure seen through skin surface temperature.

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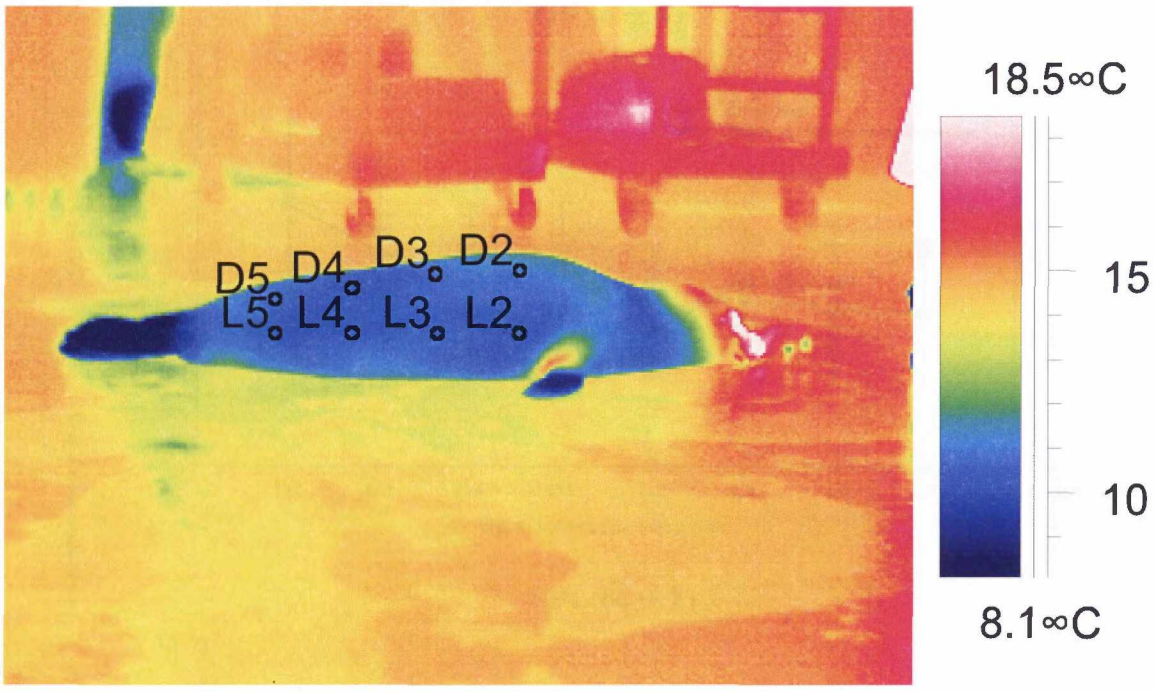

Fig. 3.1.—Thermal image depiction of the eight relative ultrasound and IRT sites on a juvenile female harbor seal (*Phoca vitulina*), PVAT via infrared thermography.

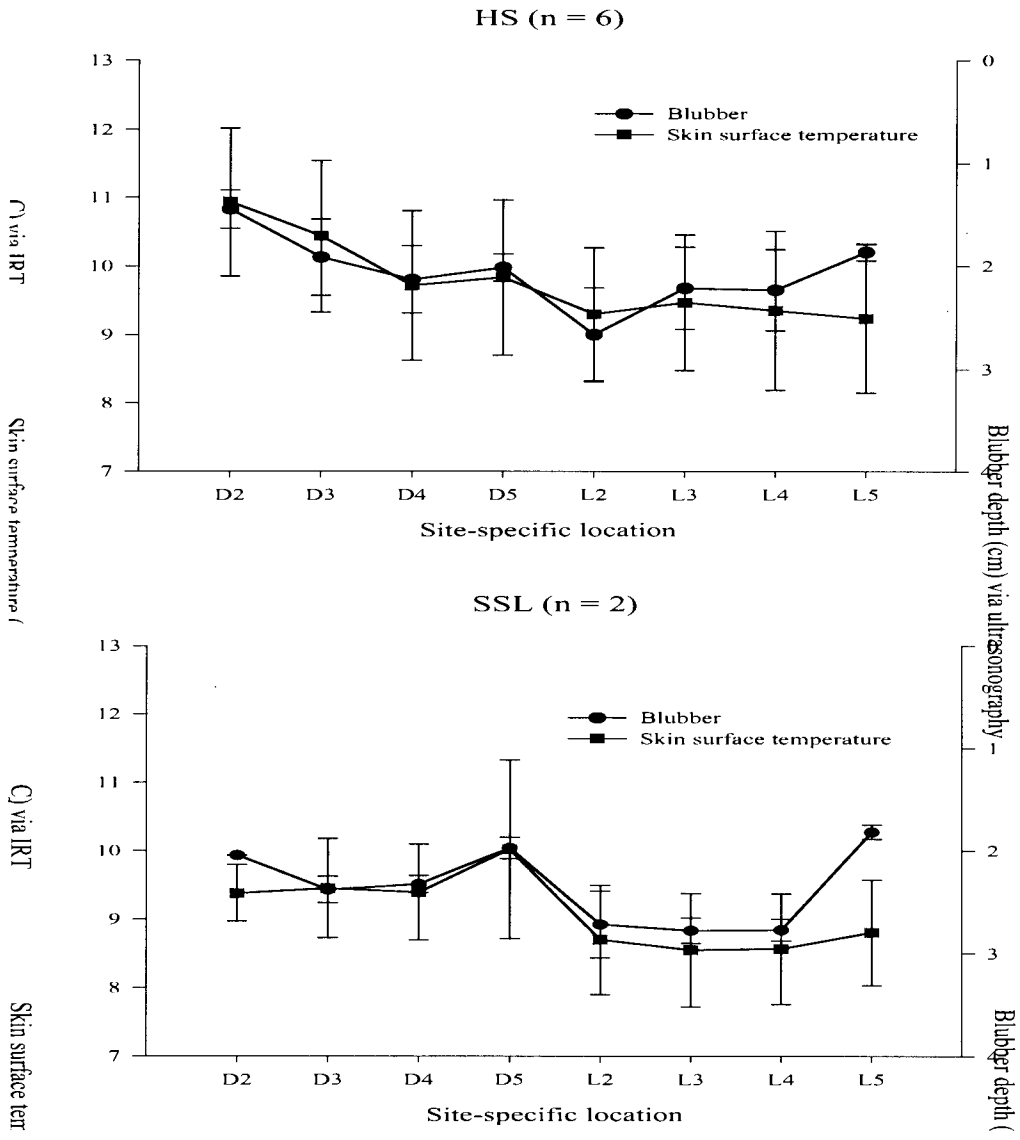

Fig. 3.2.—Annual mean skin surface temperature via IRT (left y axis) and inverse annual mean blubber depth via ultrasonography (right y axis) plotted against site-specific location. Means of each site-specific parameter (skin surface temperature and blubber depth) ± SD were found by taking the mean of 9 points per season (n = 18) per individual and then an overall site-specific mean of all individuals. Focal subjects were female juvenile harbor seals (*Phoca vitulina*) n = 6, and female adult Steller sea lions (*Eumetopias jubatus),* n = 2.

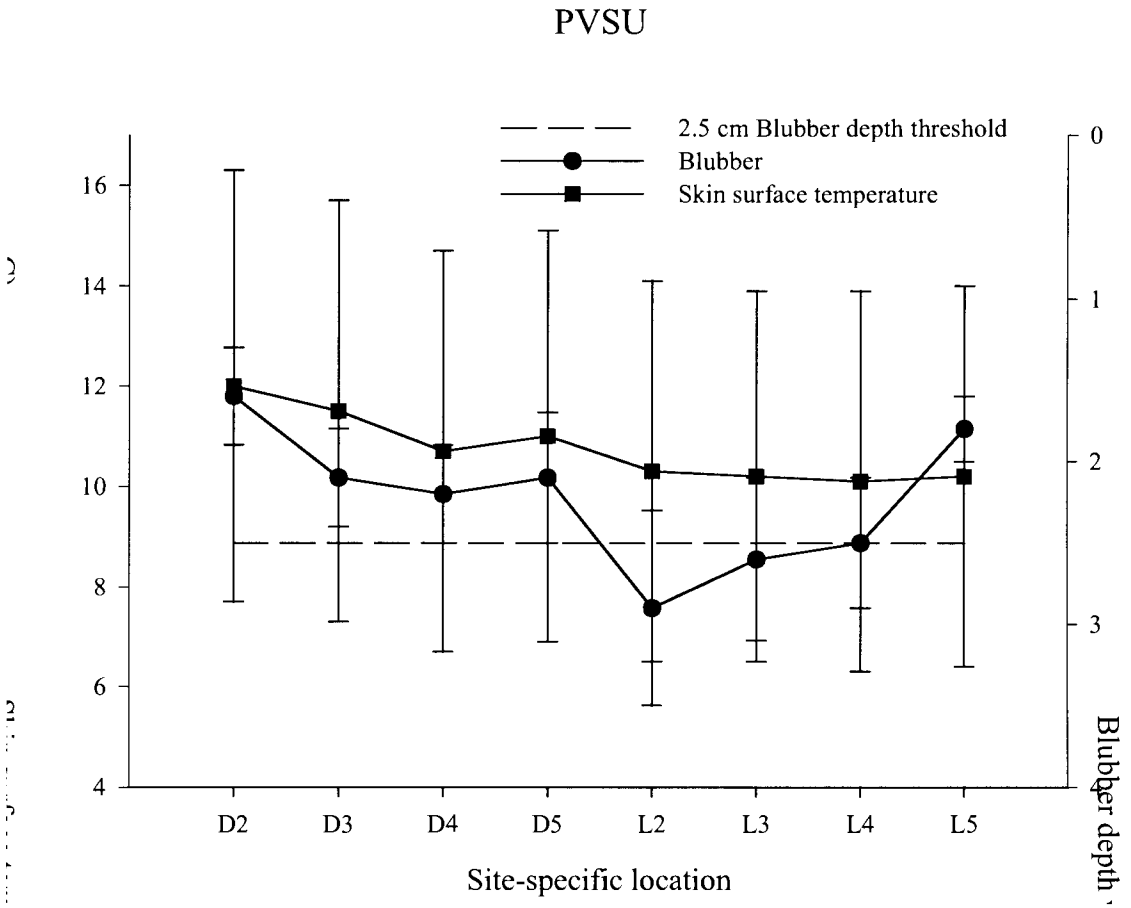

Fig. 3.3.—Annual mean skin surface temperature via IRT (left y axis) and inverse annual mean blubber depth via ultrasonography (right y axis) ± SD plotted against site-specific location on one representative individual harbor seal (PVSU). The dotted line represents the 2.5 cm blubber depth threshold where skin surface temperature will not track blubber depth at greater than 2.5 cm.

Table 3.1.—Mean seasonal variation ± SD in harbor seal (*Phoca vitulina*) skin surface temperature (°C) as measured by infrared thermography at eight sites on the body as well as annual combined mean temperature of all 8 sites and annual torso skin surface temperature. Annual coefficient of variation (COV) and non-parametric Mann-Whitney U-test is reported as COV, U-test statistic, and p-value.

Harbor seal (n = 6)	Winter	Summer	COV and Mann-Whitney U-test
IRTD2	9.1 ± 0.8	12.8 ± 1.7	12.34, 619.0, <0.001
IRTD3	8.7 ± 0.7	12.2 ± 1.8	11.97, 631.0, <0.001
IRTD4	8.1 ± 0.6	11.3 ± 1.8	11.36, 685.5, <0.001
IRTD5	8.2 ± 0.7	11.5 ± 1.8	11.32, 682.5, <0.001
IRTL2	7.7 ± 0.7	10.9 ± 1.5	10.32, 649.0, <0.001
IRTL3	7.8 ± 0.6	11.1 ± 1.6	10.64, 648.5, <0.001
IRTL4	7.7 ± 0.7	11.0 ± 1.8	11.07, 681.5, <0.001
IRTL5	7.6 ± 0.7	10.9 ± 1.7	10.83, 674.5, <0.001
IRT 8 site combined annual mean: 9.8 ± 0.6			
IRT torso annual mean: 10.0 ± 1.0			

Table 3.2.—Mean seasonal variation ± SD in Steller sea lion (*Eumetopias jubatus*) skin surface temperature (°C) as measured by infrared thermography at eight sites on the body as well as annual combined mean temperature of all 8 sites and annual torso skin surface temperature. Annual coefficient of variation (COV) and non-parametric Mann-Whitney U-test is reported as COV, U-test statistic, and p-value.

Steller sea lion (n = 2)	Winter	Summer	COV and Mann-Whitney U-test
IRTD2	8.1 ± 0.4	10.6 ± 0.4	5.46, 59.5, 0.001
IRTD3	8.1 ± 0.7	10.8 ± 0.8	5.75, 61.5, 0.001
IRTD4	8.1 ± 0.8	10.6 ± 0.6	5.59, 66.0, 0.002
IRTD5	8.9 ± 1.5	11.1 ± 1.0	5.44, 86.0, 0.027
IRTL2	7.6 ± 0.8	9.8 ± 0.8	5.32, 76.0, 0.006
IRTL3	7.4 ± 0.8	9.7 ± 0.9	5.28, 75.5, 0.006
IRTL4	7.4 ± 0.8	9.8 ± 0.8	5.70, 73.5, 0.005
IRTL5	7.6 ± 0.6	10.0 ± 0.9	5.73, 71.5, 0.004
IRT 8 site combined annual mean: 9.1 ± 0.5			
IRT torso annual mean: 9.4 ± 0.7			

Table 3.3.—Mean seasonal variation ± SD in harbor seal (*Phoca vitulina*) blubber depth (cm) as measured by ultrasound at eight sites on the body as well as the annual mean of all 8 sites. Annual coefficient of variation (COV) and non-parametric Mann-Whitney U-test column is reported as COV, U-test statistic, and p-value.

Harbor seals (n = 6)	Winter	Summer	COV and Mann-Whitney U-test
USD2	1.6 ± 0.1	1.3 ± 0.2	0.05, 2289.0, <0.001
USD3	2.1 ± 0.4	1.7 ± 0.4	0.08, 2204.5, <0.001
USD4	2.3 ± 0.3	2.0 ± 0.4	0.09, 2112.0, <0.001
USD5	2.2 ± 0.1	1.8 ± 0.2	0.09, 2420.5, <0.001
USL2	3.0 ± 0.4	2.4 ± 0.5	0.29, 2241.5, <0.001
USL3	2.5 ± 0.4	1.9 ± 0.3	0.17, 2306.5, <0.001
USL4	2.4 ± 0.4	2.0 ± 0.4	0.13, 2109.5, <0.001
USL5	2.0 ± 0.1	1.7 ± 0.1	0.07, 2329.5, <0.001
Ultrasound 8 site combined annual mean: 2.1 ± 0.4			

Table 3.4.—Mean seasonal variation ± SD in Steller sea lion (*Eumetopias jubatus*) blubber depth (cm) as measured by ultrasound at eight sites on the body as well as the annual mean blubber depth of all 8 sites. Annual coefficient of variation (COV) and non-parametric Mann-Whitney U-test column is reported as COV, U-test statistic, and p-value.

Steller sea lions (n = 2)	Winter	Summer	COV and Mann-Whitney U-test
USD2	2.1 ± <0.1	2.0 ± <0.1	0.06, 194.0, 0.311
USD3	2.4 ± 0.2	2.3 ± 0.1	0.10, 187.0, 0.429
USD4	2.4 ± 0.1	2.2 ± 0.1	0.15, 222.0, 0.058
USD5	2.0 ± 0.2	1.9 ± 0.1	0.17, 190.0, 0.376
USL2	2.9 ± 0.4	2.6 ± 0.3	0.27, 242.0, 0.011
USL3	2.8 ± 0.1	2.7 ± 0.1	0.13, 183.5, 0.496
USL4	2.9 ± 0.1	2.6 ± 0.1	0.15, 216.0, 0.087
USL5	2.0 ± 0.2	1.6 ± <0.1	0.21, 249.5, 0.006
Ultrasound 8 site combined annual mean: 2.4 ± 0.4			

Table 3.5.—Annual site-specific relationships between skin surface temperature (x) and blubber depth (y) using simple linear regression in the harbor seal *Phoca vitulina* (HS, n = 6) and the Steller sea lion *Eumetopias jubatus* (SSL, n = 2).

Site	Species	R^2 value	*P* value	Equation
D2	HS	0.338	0.047	y = 2.146 - 0.060x
	SSL	0.486	0.303	y = 2.242 - 0.023x
D3	HS	0.268	0.085	y = 2.880 - 0.091x
	SSL	0.453	0.327	y = 2.945 - 0.058x
D4	HS	0.419	0.023	y = 3.251 - 0.113x
	SSL	0.992	0.004	y = 3.127 - 0.083x
D5	HS	0.480	0.013	y = 2.835 - 0.082x
	SSL	0.152	0.610	y = 1.742 + 0.023x
L2	HS	0.255	0.094	y = 3.975 - 0.138x
	SSL	0.020	0.859	y = 2.949 - 0.029x
L3	HS	0.345	0.045	y = 3.501 - 0.138x
	SSL	0.070	0.735	y = 2.972 - 0.023x
L4	HS	0.331	0.050	y = 3.308 - 0.114x
	SSL	0.310	0.443	y = 3.309 - 0.062x
L5	HS	0.692	0.001	y = 2.524 - 0.071x
	SSL	0.628	0.208	y = 2.908 - 0.126x

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SYNTHESIS

As the polar climate is shifting at increasing rates, sensitive populations will also be increasingly affected in their physiology and spatial distribution. Therefore, non-invasive methods to monitor populations and collect data will become important techniques to use in the field to limit disturbance in sensitive populations as research permit restrictions become stronger. Infrared thermography (IRT) is one potential technique that has been around since the early 1900s, but its use with many species is still limited due to lack of availability of controlled validation and baseline studies. Longitudinal data are more useful when doing such validations as surface temperature patterns will change depending on environmental variables, age of the population, and seasonal changes of mature individuals.

In this thesis, IRT was validated for use in harbor seals and Steller sea lions to provide a baseline in regional and seasonal skin surface temperatures, and to further determine if the data collected from this method may be used as a proxy for condition estimates (i.e., vary predictably with blubber depth) in pinnipeds. In Chapter 2 (Thermal windows in seals and sea lions: what's hot and what's not?), it was found that when comparing two objects of differing emissivities at increasing known temperatures, IRT-based temperature values are within absolute accuracy of the two methods and both have a highly linear relationship to one another. Manipulation of object parameters within the software for temperature output had minimal to no effect on surface temperature output seen in this emissivity range (0.96 – 1.00). Assessment of consistent hot and cold spots showed that skin surface temperature variation was more apparent in the harbor seals (n = 6) than in the Steller sea lions (n = 2). Fluctuations in regional skin surface temperature occurred between the winter (Oct – Apr) and reproductive (May – Jul) seasons, and then again between the molt (Aug – Sep) and winter. While ten baseline thermal regions were identified, four of these regions were determined to be possible thermal windows, or an active area of heat dissipation and/or retention (shoulder, axillae, fore and hind flippers). The goals of Chapter 3 (The thick and thin of body condition: does blubber depth influence skin surface temperature in pinnipeds?), were to assess the relationship of skin-

surface temperature via IRT to blubber depth via ultrasound. While blubber depth did account for some variation in skin surface temperature (i.e., 69% at L5 in harbor seals, 99% at D4 in sea lions), there are clearly other outstanding factors also at play (e.g., ambient air temperature, physiological state). If these additional factors can be quantitatively accounted for, it is conceivable that body condition could be estimated from IRT-based skin surface temperature measurements.

Appendix A. Seasonal changes (Winter (W), Reproductive (R), Molt (M)) of surface temperatures captured via infrared thermography on six juvenile female harbor seals (*Phoca vitulina*) and two adult female Steller sea lions (*Eumetopias jubatus*) from February 2007 – January 2008. Mann-Whitney U test where significance is > 95%.

	Harbor seals (*N* = 6)			*Steller sea lions* (*N* = 2)		
	W – R U statistic p-value	R – M U statistic p-value	M - W U statistic p-value	W – R U statistic p-value	R - M U statistic p-value	M – W U statistic p-value
Body	U = 166.0 p = 0.012	U = 209.5 p = 0.105	U = 106.0 p < 0.001	U = 17.5 p = 0.128	U = 31.5 p = 0.958	U = 18.0 p = 0.141
Torso	U = 156.0 p = 0.006	U = 209.5 p = 0.105	U = 105.0 p < 0.001	U = 11.0 p = 0.027	U = 39.5 p = 0.430	U = 18.5 p = 0.155
Head	U = 191.0 p = 0.045	U = 196.0 p = 0.058	U = 122.0 p = 0.001	U = 19.5 p = 0.188	U = 36.0 p = 0.673	U = 24.0 p = 0.397
Eye	U = 236.0 p = 0.238	U = 266.0 p = 0.065	U = 204.0 p = 0.083	U = 17.0 p = 0.115	U = 28.0 p = 0.674	U = 14.0 p = 0.059
Muzzle	U = 219.5 p = 0.158	U = 175.0 p = 0.020	U = 115.5 p < 0.001	U = 28.0 p = 0.674	U = 23.0 p = 0.344	U = 20.0 p = 0.207
Shoulder	U = 84.5 p < 0.001	U = 236.0 p = 0.283	U = 65.5 p < 0.001	U = 8.5 p = 0.014	U = 42.5 p = 0.270	U = 13.0 p = 0.046
Axillae	U = 149.5 p = 0.004	U = 219.0 p = 0.155	U = 113.0 p < 0.001	U = 18.0 p = 0.140	U = 31.0 p = 0.916	U = 20.0 p = 0.207
Hip	U = 91.5 p < 0.001	U = 256.5 p = 0.516	U = 66.0 p < 0.001	U = 12.0 p = 0.036	U = 41.0 p = 0.344	U = 15.5 p = 0.083
Fore Flipper	U = 148.0 p = 0.004	U = 218.5 p = 0.152	U = 88.5 p < 0.001	U = 10.5 p = 0.024	U = 41.0 p = 0.344	U = 18.0 p = 0.141
Hind Flipper	U = 198.5 p = 0.065	U = 222.5 p = 0.177	U = 107.5 p < 0.001	U = 16.0 p = 0.093	U = 32.0 p = 1.000	U = 17.5 p = 0.127